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NUTRITION REVIEWS

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Nutrition and Pediatrics 289
Present Knowledge of Iron and Copper in Human Nutrition 291
Effect of Thiamine Deprivation upon Gastric Secretion in Rats 293
Synthesis of Fatty Acids in the Animal Body 295
Thyroid Activity and Poultry Nutrition 297
Caloric Intakes of a Group of Boston Children 298
Supplementation of the Diet of Aircraft Workers 300
Relation of the Kwashiorkor Syndrome to Ariboflavinosis 303
Pellagra 304
Methionine Intake and the Rate of Transmethylation 306
Tooth Decay in the Cotton Rat 308
Regeneration of Liver Protein as a Test for Dietary Protein Adequacy 309
Biosynthesis of the Vitamin B-Complex and Human Nutrition 310
Pre-exercise Nutriment and Physical Efficiency 313
Ascorbic Acid Content of Blood and Plasma in Normal Rats 314
Interpretation of Bacterial Growth Inhibition and its Reversal 315
Nutritional Edema 317
Notes 319

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NUTRITION REVIEWS

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NUTRITION AND PEDIATRICS

Pediatricians have become accustomed to think of nutrition as one of the important factors determining the health, growth, and maturation of infants and children. While much has been published about the role of the vitamins, protein, and minerals, certain of the broader relations between nutrition and pediatrics have received relatively little attention.

Although an intake of building materials such as protein and minerals corresponding to that recommended by the National Research Council provides an ample margin of safety, it is not at all clear whether such generous allotments are necessary requisites to the maintenance of good health and to satisfactory growth and maturation. There are scattered pieces of evidence which suggest that because the organism is able to adapt to a limited intake by increased efficiency of absorption and utilization, much smaller quantities of protein, minerals, and vitamins may suffice (H. H. Mitchell, *J. Am. Dietet. Assn.* **20,** *511 (1944)*). For example, while the N.R.C. recommends that children have a protein intake of about 2 g. per kilogram per day, there is evidence that they can grow when receiving as little as 0.7 g. per kilogram daily (W. M. Bartlett, *Am. J. Dis. Child.* **32,** *641 (1926)*). This latter value bears an interesting relation to the approximate minimal adult maintenance requirements of protein (0.5 g. per kilogram per day) and the calculated daily protein increment of the growing child (about 0.1 g. per kilogram per day) (N. B. Talbot and E. H. Sobel, "Factors Determining the Growth of Children, Advances in Pediatrics," *Vol.* **2,** *Interscience Publishers, New York (in press)*). Again the daily calcium balance during the major part of childhood usually amounts to less than + 175 mg. (A. T. Shohl, "Mineral Metabolism," *Reinhold Publishing Co., New York (1939)*). If, as has been reported, children adapted to a low calcium intake can absorb and retain as much as 80 to 90 per cent of the calcium intake (Mitchell, *loc. cit.*), the N.R.C. recommended daily intake of 1 g. of calcium may be considerably in excess of minimum requirements. Though these thoughts are not intended to imply that the N.R.C. allotments are undesirable or harmful, they do suggest that protein or calcium intakes appreciably lower than the N.R.C. values may not be injurious. Since the production of animal protein foods is a very expensive process in terms of the total calories required to keep animals alive while they are growing, it would be of practical value in a partially famished world to know the absolute protein requirements.

The utilization of protein eaten is influenced in part by the total caloric intake. That is, there is the well known tendency for protein to be catabolized if the individual is in negative caloric balance. This is not an obligatory phenomenon, however, for protoplasmic growth can occur provided there is an adequate intake of building materials even though the caloric content of the organism may decrease (H. Aron, *Biochem. Z.* **30,** *207 (1911)*). Under such conditions of negative caloric balance and protein anabolism, the caloric deficit is made up by the combustion of body fat stores. In this connection it is of interest that during the middle period of childhood when the increment of protoplasm amounts to about 2 kg. per year, the caloric equivalent of that protoplasm could be provided by the combustion of less than 0.3 kg. of body fat. A normal child of 20 kg. has about 2 to 3 kg.

of body fat. The fact that a negative caloric balance does not necessarily result in a cessation of growth (i.e. protoplasmic anabolism or positive nitrogen balance) suggests that the caloric balance influences protein metabolism indirectly.

This introduces the fact that certain hormones are known to exert a powerful influence upon the fate of available protein (or amino acids). These hormones are of two chief types: (a) anabolic substances such as the pituitary growth hormone and the testicular androgens; (b) antianabolic or catabolic agents such as certain 11-oxycorticosteroids (the "S" or gluconeogenetic hormone of F. Albright (*Harvey Lectures* **38,** *123* (*1942*)). That these substances bear an important relation to nutrition is illustrated by the following types of observation. Hypophysectomized animals undergo protoplasmic growth only very slowly even when the dietary intake of calories and building materials is more than sufficient for maintenance. Under these circumstances the protein ingested in excess of minimal needs tends to be catabolized while any surplus calories are stored as body fat (Talbot and Sobel, *loc. cit.*). On the other hand, in the presence of a protein anabolic hormone such as testosterone, growth may be induced in children suffering from an inadequate caloric intake (Talbot and Sobel, *loc. cit.;* N. B. Talbot *et al.*, *Am. J. Dis. Child.* **69,** *267* (*1945*)). Similar effects have been observed in normal young adults subjected to six day periods of absolute fasting (A. Butler *et al.*, *J. Clin. Endocrinology* **5,** *327* (*1945*)). In them testosterone caused an appreciable diminution in the negative nitrogen balance. This was accompanied by a tendency to lower fasting blood sugar values (indicating decreased gluconeogenesis from protein) and to a much larger excretion of organic acids in the urine (presumably reflecting an increased catabolism of body fat to compensate for the diminished catabolism of body protein under conditions of fasting). Still another example is provided by observations on the influence of the aforementioned adrenal cortical "S" hormones. These are produced in increased quantities by previously well nourished and healthy persons when they become severely injured as by an extensive burn (H. Selye, *J. Clin. Endocrinology* **6,** *117* (*1946*)). They are also produced in excessive quantities by patients with Cushing's syndrome (Albright, *loc. cit.*). Such individuals either show a strongly negative nitrogen balance or fail to develop a positive nitrogen balance under ordinarily favorable nutritional circumstances. That is, protein anabolism tends to be blocked when there is an excess of these agents. In other words, hormones occupy a position almost of equal importance to the diet in the determination of body protoplasmic changes. Hence it seems that variations in the rates of growth and maturation of children apparently occasioned by variations in diet may in the final analysis reflect a physiologic adaptation by the endocrine system to the dietary intake. Available information suggests that an excessive dietary intake (obesity) ordinarily tends to promote slightly increased pituitary activity as evidenced by accelerated growth, skeletal and sexual maturation (H. Bruch, *Am. J. Dis. Child.* **58,** *457* (*1939*)), while an inadequate intake frequently has the opposite effect (N. B. Talbot *et al.*, *read before the Am. Pediatric Soc., May, 1946*). That these are not obligatory relationships, however, is indicated by the fact that there are short, retarded obese children as well as tall, advanced thin children.

Of further interest are the causes of dietary discrepancies. While famines, poverty, food habits, and various diseases may explain nutritional deviations in some cases, it is difficult to find an explanation in others. In many cases of obesity it has been possible to link the tendency toward dietary over-indulgence with emotional factors (H. Bruch, *Am. J. Dis. Child.* **59,** *739* (*1940*)). The opposite reaction

to emotional disturbances, namely anorexia, may be responsible for many instances of inadequate nutrition in children.

Therefore, an evaluation of the part played by nutrition in health and disease would appear to require knowledge of the physical, physiologic, emotional, and environmental status of the individual. Both for practical and theoretical reasons more detailed information concerning the absolute minimal requirements of the essential nutrients and of the influence of nutrition upon metabolic regulators should aid greatly in defining precisely the role of nutrition in pediatrics.*

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PRESENT KNOWLEDGE OF IRON AND COPPER IN HUMAN NUTRITION

Iron is found in the body in hemoglobin (57 per cent), in storage depots (20 per cent), in various important enzymes ("parenchyma iron," 16 per cent) and in the myoglobin of muscles (7 per cent) (P. F. Hahn, *Medicine* **16,** *249 (1937)*). The body of an adult human being contains somewhere between 3 and 5 g. of iron. All nuclei of animal cells are said to contain this element. The iron present in the liver, spleen, and bone marrow can probably be used for blood formation. Since hemoglobin plus storage iron taken together make up 77 per cent of the body iron, approximately three fourths of the iron is concerned in hematopoiesis.

Extremely little iron is excreted in the urine but there is a low though constant amount to be found in the bile. Since the iron derived from the destruction of red cells is available for hematopoiesis, one would expect to find that a need for external sources of iron becomes manifest largely or exclusively when blood is being lost through hemorrhage (physiologic or pathologic), or when requirements for growth or reproduction exist which make demands beyond the capacity of the stores of iron within the body. It has been stated that the daily minimum intake of iron is 5 to 17 mg. (R. M. Leverton, *J. Nutrition* **21,** *617 (1941)*) but these figures cannot be regarded as exact. It is true, no doubt, that the requirement for the female is much greater than that of the male, possibly four or more times as great. The requirement for growing children may be as great as 0.76 mg. per kilogram of body weight (K. D. Blackfan, "Report of the White House Conference on Child Health and Protection," *Century Co. (1932)*).

Eggs, molasses, liver, beef muscle, and dried fruits are good food sources of iron. The well known alpha dipyridyl test for the "availability" of iron in food depends on the fact that this substance reacts with ionizable ferrous iron but not with hematin or other forms of organically bound iron. However, close correlation between availability as measured in this way and physiologic activity has not always been found.

The hydrochloric acid of the gastric juice dissolves the iron, and in the presence of achlorhydria the assimilation of food iron is diminished. The presence of reducing substances in the diet such as ascorbic acid is thought to aid absorption by preventing oxidation of ferrous iron. Man absorbs ferrous iron more readily than ferric (*Nutrition Reviews* **3,** *110 (1945)*). Other factors in the gastrointestinal tract may also influence the absorption of iron. These include bile pigment, the ratio of calcium to phosphorus, and perhaps the gastric mucin.

It is claimed that the intestine can itself control the amount of iron absorbed. This hypothesis is based largely on experiments

in which radioactive iron was fed and its appearance in the red cells of the recipient noted (*Nutrition Reviews* **1,** *154* (*1943*)). Patients in whom the iron stores were presumably high (pernicious anemia, hemolytic anemia, polycythemia) were found to pick up very small amounts of the given dose of radioactive iron in their red cells, whereas the red cells of persons with poor stores of iron took up a much larger proportion of the radioactive iron. Unfortunately, these observations did not take into account the factor of rate of hemoglobin production; obviously, in normal persons the rate of hemoglobin production would be expected to be less than in patients who are anemic as the result of iron deficiency. Still less radioactive iron would be expected to be taken up by the red corpuscles of persons in whom hemoglobin formation was limited by some deficiency as, for example, lack of antipernicious anemia principle. In persons with pernicious anemia it is well known that iron cannot be utilized efficiently for in patients dying of this disease the tissues are found to contain large amounts of iron. A similar condition exists in pyridoxine deficiency anemia.

It has been postulated that reserve stores of iron in the body control iron absorption, exerting their effect on the gastrointestinal tract via the plasma iron level. In the mucosa an "iron acceptor" compound may exist whose iron saturation and consequent capacity for iron absorption is controlled by the plasma iron. The reverse procedure, namely passage of iron from the plasma via the "acceptor" compound to the bile is prevented by the fact that the plasma iron is combined with protein. S. Granick (*Science* **103,** *107* (*1946*)) has suggested that iron absorbed into the mucosal cells in the ferrous form is stored there in the form of ferritin, which is a combination of the protein apoferritin with ferric hydroxide. In the cell there may be an equilibrium between ferrous iron and the ferric iron stored in ferritin. A lowering of the concentration of iron in the plasma may lead to the removal of ferrous iron from the mucosal cells and diminution of ferritin iron in the mucosa. When this has reached the point of physiologic unsaturation more iron is absorbed. It may be expected that further investigation will bring support for or disprove this hypothesis. It is not consistent with the observation that pyridoxine deficient animals though unable to use iron continue to absorb it when it is fed and that the characteristic high plasma iron in pyridoxine deficient animals can be prevented only by omitting iron from the diet (*Nutrition Reviews* **3,** *29* (*1945*)).

When iron is absorbed it is probably carried by the blood plasma either to the liver, spleen, or other organs for storage or to the bone marrow for hemoglobin synthesis. The content of iron in the plasma probably represents the balance between the iron being absorbed, that passing to the bone marrow from the liver, and that coming from the breakdown of red cells (C. V. Moore, C. A. Doan, and W. R. Arrowsmith, *J. Clin. Invest.* **16,** *627* (*1937*)). Increasing attention is being given to the plasma iron content in various disease states and such studies may yield new information concerning the metabolism of iron in man. It has been known for some time that in states of iron deficiency the plasma iron content is low, whereas in pernicious anemia and in pyridoxine deficiency anemia, the plasma iron is high. It was recently shown (G. E. Cartwright and co-workers *Ibid.* **25,** *65* (*1946*)) that in association with infection the plasma iron falls and remains low so long as the infection exists. In this study the fallacy of drawing conclusions concerning iron absorption from plasma iron levels following feeding of iron was shown. When iron was given by mouth the plasma iron content increased relatively little. That this was not due to a failure to absorb iron but rather was attributable to rapid removal of iron

from the plasma, was indicated by the speed with which iron disappeared even following the intravenous injection of iron.

Much needs to be learned concerning the intermediate metabolism of iron. Little is known regarding the chemical nature of stored iron or concerning the form in which it is used by the bone marrow for hemoglobin synthesis. The investigation of the pathogenesis of the anemia of infection indicates that in the presence of infection a fault in the intermediate metabolism of iron may develop.

Iron deficiency in man is recognized generally by the presence of hypochromic anemia. The clinical syndrome associated with this type of anemia is characterized, however, by changes in addition to those found in the blood: striking alterations in the finger nails which become lusterless, flattened, and even concave ("spoon nails"); wrinkling and dryness of the skin; difficulty in swallowing; papillary atrophy of the tongue; and fissures at the corners of the mouth. As was discussed previously (*Nutrition Reviews* **4**, *263* (*1946*)) all of these changes may be due to lack of iron alone rather than the result of associated vitamin B deficiency.

The role of copper in human nutrition is still obscure. While there is considerable evidence that copper plays a role in erythropoiesis this evidence is based largely on experiments in animals. Observations in man are not clearcut, perhaps because copper deficiency is so rare. In infants with nutritional anemia, several investigators have noted an accelerated or a more continued response to iron when copper was added in a ratio of about 1 to 25 than when iron was given alone (for example, J. H. Hutchison, *Quart. J. Med.* **7**, *397* (*1938*)). In several instances in infants with hypochromic anemia the administration of copper produced a reticulocyte response after ferrous sulfate had already caused a significant rise (C. V. Moore, H. Q. Bierman, V. Minnich, and W. R. Arrowsmith, "Blood, Heart, and Circulation," *p. 34, Science Press* (*1940*)). In older children and adults, however, the evidence is wholly inconclusive and there are several studies which have failed entirely to indicate a need for copper.

EFFECT OF THIAMINE DEPRIVATION UPON GASTRIC SECRETION IN RATS

Thiamine deficiency is associated occasionally with loss of appetite and vomiting, and it is thought that thiamine is in some way essential for the maintenance of normal gastric secretion. Examination of the data concerning gastric secretion, however, leaves one in doubt as to the basis for this view. Many of the studies were made in man and in animals at a time when the crystalline B-vitamins were not available in pure form. To refer only to the more recent experiments in man, it can be mentioned that W. C. Alvarez and associates (*Am. J. Digest. Dis.* **3**, *102* (*1936*)) observed no change in the acidity or the pepsin concentration of the gastric juice of 2 persons fed a diet markedly deficient in thiamine for a period of six weeks. R. D. Williams, H. L. Mason, and B. F. Smith (*Proc Staff Meetings Mayo Clinic* **14**, *787* (*1939*)) placed 4 young women on a diet containing less than 0.1 mg. of thiamine chloride per day for twenty weeks. A low level or an absence of free gastric acidity was reported at the end of that period of time but figures for the acidity before, during, and after the experimental period were not given and no information was supplied concerning the method of gastric analysis.

H. Shay, S. A. Komarov, M. Gruenstein, and S. S. Fels (*Gastroenterology* **6**, *199* (*1946*)) tested the problem under better controlled conditions than had been done before. Rats were used in their experiments and two types of diets were fed. In one group the vitamin

B factors were supplied in the form of autoclaved yeast as had been done in experimental studies reported previously; in the other group only the known crystalline B supplements, with the exception of biotin and "folic acid," were given. Since loss of appetite and weight loss are two of the cardinal signs of thiamine depletion, two additional groups of animals were included. In these the total food intake was restricted so that the weight loss approximated that of the thiamine deprived groups but the daily yeast or crystalline vitamin supplement was maintained at the same level as in the control groups.

Two treated animals and 2 corresponding control animals were always studied at the same time by a procedure designed to evaluate the spontaneous secretion of gastric juices. The animals were fasted for eighteen hours, except the thiamine depleted ones which were obviously too weak to undergo such a rigid preparatory period and were operated upon without preliminary fasting. In all instances, under urethane anesthesia, a gastric fistula was made, the pylorus was ligated, and a small segment of the cecal wall was exteriorized according to a technic previously described by these investigators. The urethane anesthesia was continued throughout the period of observation which lasted from thirty-six to seventy-two hours. The gastric secretory activity was studied routinely by collecting hourly specimens of gastric secretion. To maintain water and electrolyte balances normal saline and isotonic hydrochloric acid-saline solution were injected into the cecum at regular intervals.

Following operation, stabilization of the gastric secretory activity occurred in two to four hours. Samples were considered representative only after stabilization had taken place. When several samples had been collected the gastric fistula was stoppered securely and the animal was left overnight under deep urethane anesthesia. The following morning the gastric fistula was opened and the night's secretion was collected. Warm saline and acid were then injected into the cecum until a copious excretion of urine occurred. The rate of gastric secretion was calculated per unit body weight. In several hundred experiments on normal rats, these investigators have found that the rate of secretion is similar in animals of vastly different weights.

The experiments showed that in acute severe thiamine depletion, when the animals had lost 25 to 30 per cent of their initial body weight, the volume of spontaneous gastric secretion was much greater than in the corresponding control rats. The acidity, peptic power, and total chloride concentration, however, were not altered. That the hypersecretion was related entirely to the thiamine depletion was indicated by the fact that a similar increase in secretion was not observed in control animals receiving restricted diets. It was noted that in the advanced stages of thiamine depletion a dissociation of the elements of gastric secretory function occurred. The volume of the secretion was increased and the peptic power of the gastric juice remained within normal limits but the acidity decreased. Similar changes were observed in the rats fed autoclaved yeast as a source of B-vitamins and in those given crystalline vitamins with the exception of thiamine.

Since there is nothing in the literature to indicate that thiamine depletion may result in hyperactivity of the gastric glands, the investigators were unprepared to find, as they regularly did, that the fasting stomach was in a state of hyperactivity in rats suffering from thiamine depletion. This unexpected finding might be attributed to the experimental procedure were it not that, in control animals operated upon in the same way as the experimental animals, the results differed strikingly from the observations in the thiamine deficient ones. Perhaps an increased gastric secretion under the influence of thiamine deficiency has been overlooked. An increase in gastric secretion may possibly explain the striking distention of the stomach which has been observed in

thiamine deficient animals (M. M. Wintrobe and co-workers, *Bull. Johns Hopkins Hosp.* **71,** *141 (1942)*).

An incidental observation which is of some interest because of the reported high incidence of peptic ulceration in the presence of protein deficiency (*Nutrition Reviews* **4,** *278 (1946)*) was the finding that gastric ulceration occurred in rats on a calorically restricted diet, whether it was adequate or deficient in thiamine. The incidence, number, and severity of the lesions, however, were much greater in the thiamine depleted animals than in those receiving adequate amounts. Thus, all of the thiamine deprived rats (32) exhibited severe gastric ulcerations in the body of the stomach, whereas gastric ulceration was observed only in half the control animals which had been on a calorically restricted diet. In the control animals which had been on a calorically unrestricted diet no ulceration whatever was observed. The investigators suggest that the increased rate of secretion of an active gastric juice may be a factor which operates to make the ulceration which develops in association with dietary restriction more severe.

SYNTHESIS OF FATTY ACIDS IN THE ANIMAL BODY

The mechanism of the biologic synthesis of fats from carbohydrate, despite its importance from a nutritional and biochemical viewpoint, is still very obscure. It has been postulated that hexose units themselves might be condensed to form the carbon skeleton of fatty acids, but there is little evidence for this mechanism. A working theory of fatty acid anabolism should account for the exclusive occurrence in nature of fatty acids with an even number of carbon atoms, and according to T. P. Hilditch ("The Chemical Constitution of Natural Fats," *p. 281, Wiley, N. Y. (1941)*) also for the quantitatively important oleic acid with its centrally located double bond. The former requirement has supported the belief that two-carbon carbohydrate residues may be the building stones for fat synthesis, and one of the common theories has been the combining of acetaldehyde units by an aldol type condensation. Such a reaction is common in the organic chemistry laboratory, but has not been adequately demonstrated in biologic systems. However, such a mechanism would be expected to lead to aldehydes of the higher fatty acids, and such compounds have indeed been isolated from animal tissue (G. Ehrlich and H. Waelsch, *J. Biol. Chem.* **163,** *195 (1946)*). These workers have investigated the possible role of these aldehydes in fatty acid metabolism by measuring their deuterium content as well as that of fatty acids and other lipid fractions after enriching the body fluids with deuterium oxide. Although the higher fatty acid aldehydes contained appreciable amounts of deuterium, their content was invariably less than that of the fatty acid fractions, and the investigators conclude that these aldehydes are not involved in the over-all synthesis or transport of fatty acids but are concerned with some special aspect of fatty acid metabolism.

Studies on the synthesis of fats by microorganisms have generally, but not invariably, shown that alcohol and acetic acid are good sources of fat. Only within the past two years has there been adequate evidence that acetic acid is a direct source of fatty acids in the animal body. D. Rittenberg and K. Bloch (*J. Biol. Chem.* **154,** *311 (1944)*; **160,** *417 (1945)*) have applied the use of labeled carbon and hydrogen in studying the synthesis of fatty acids from acetic acid in rats and mice. In their experiments with rats, acetate containing almost 20 per cent excess C^{13} in the carboxyl group was employed. The fatty acids of the carcass were isolated, and there was found twice as great an excess of C^{13} in the carboxyl group

as in the whole molecule. In the experiments with mice, acetate containing C^{13} in the acid group and a 77 per cent excess of deuterium in the methyl group was fed. The fatty acids isolated contained an excess of both C^{13} and deuterium. The excess C^{13} of the expired carbon dioxide at the end of the experiment was found to be nearly identical with that of the complete experimental diet, and since the C^{13} excess in the fatty acids was higher, the use of carbon dioxide for fat synthesis could not account for the facts.

These experiments indicate that the labeled carbon of the acetate carboxyl occurs in alternate atoms of the fatty acid molecule, and furthermore, due to the deuterium excess found, that both carbons of acetic acid are used in the synthesis. The evidence does not prove that this is the only method by which fatty acids can be synthesized. Based on earlier data (K. Bloch and D. Rittenberg, *J. Biol. Chem.* **159,** *45 (1945)*) obtained on the dilution of labeled acetate fed at the same level, it was calculated that at least one quarter of all carbon atoms of the fatty acids were derived from the acetate fed.

In attempting to trace the individual reactions involved in the synthesis of fatty acid from acetic acid, it seems probable that formation of acetoacetic acid is a first step. A. L. Lehninger (*J. Biol. Chem.* **148,** *393 (1943)*) has described an enzyme which catalyzes the formation of acetate from acetoacetate, and G. Medes, S. Weinhouse, and N. F. Floyd (*Ibid.* **157,** *751 (1945)*) have demonstrated by isotope dilution technic that acetate is readily and to a considerable extent converted to acetoacetate. Evidence has accumulated that the principal pathway of acetoacetate metabolism is by condensation with oxaloacetate to form members of the tricarboxylic acid cycle (see J. M. Buchanan, W. Sakami, S. Gurin, and D. W. Wilson, *Ibid.* **159,** *695 (1945)* and G. Medes, N. F. Floyd, and S. Weinhouse, *Ibid.* **162,** *1 (1946)*). These studies greatly broaden our understanding of both carbohydrate and fat catabolism, but make it more difficult to classify acetoacetate as a characteristic intermediate of fat synthesis without demonstration of its conversion to fatty acids containing more carbon atoms.

It would be logical to suppose that either acetoacetate itself, or the butyric acid arising from it by reduction, might condense with a further molecule of acetate to form a keto (beta, delta, beta) caproic acid. From the standpoint of animal metabolism the evidence is lacking to postulate this step. The secretion of caproic acid in goat's milk is suggestive, although it is of course possible that this caproic acid arises by degradation of higher fatty acids, rather than by the type of synthesis suggested.

As is frequently the case, bacteria often present specialized or limited metabolic steps which may be obscured in other tissues by a more complex metabolism. Thus in the case of animal metabolism it has been impossible as yet to observe the several intermediates which must be involved in the breakdown or formation of higher fatty acids, but in the case of Clostridium Kluyveri it has been possible to isolate butyric and caproic acids as products of the metabolism of acetate and ethyl alcohol (H. A. Barker, M. D. Kamen, and B. T. Bornstein, *Proc. Soc. Nat. Acad. Sci.* **31,** *373 (1945)*). When this organism was grown anaerobically in a medium containing alcohol and carboxyl labeled acetate (C^{14}), the butyric acid isolated had C^{14} almost equally distributed between the carboxyl group and the beta carbon. The caproic acid had one third of its C^{14} located in the carboxyl group. Alcohol appeared to be oxidized to acetic acid, this oxidation apparently being required for the condensation reactions. In further experiments, H. A Barker and co-workers isolated labeled caproic acid as a product of the incubation of carboxyl labeled butyric acid and alcohol. The C^{14} did not occur in the carboxyl group of caproic acid, but appeared to result from the condensation of the carboxyl group of butyric acid with the methyl group of acetate (arising from alcohol). This is the same type of reaction by which aceto-

acetic acid is formed from two acetate molecules.

There is thus increasing evidence that higher fatty acids are synthesized by successive condensations of acetate. Whether the various intermediates are first reduced to saturated fatty acids before further condensation or complex keto acids are formed, is not yet known. Certainly the synthesis of fatty acids in the animal body offers a fruitful though difficult biochemical field, and one of great significance to the better understanding of nutrition.

THYROID ACTIVITY AND POULTRY NUTRITION

The relation of endocrine secretions to growth and reproduction in animals has attracted considerable attention during recent years. The thyroid hormone has received special study in relation to its effects on the chicken because of the importance of reproductive rate in commercial flocks. The results obtained are of interest, not only to poultry breeders, but also to endocrinologists and nutritionists.

Some of the earliest experiments on thyroid feeding to chickens were carried out by F. A. E. Crew (*Proc. Roy. Soc. Edin.* **45,** *252 (1925)*). He observed that fowls 5 to 8 years of age were remarkably rejuvenated by the administration of desiccated thyroid over a period of six months. The birds molted during the early part of the treatment, and the new plumage resembled that of younger birds. Comb and wattles became livid, and egg production rose from 6.67 eggs per hen for the previous six months to 34 eggs per hen during the treatment, and 24 eggs for the six months following the cessation of the experiment.

Large scale extension of these observations was made possible by E. P. Reineke and C. W. Turner (*Mo. Agr. Exp. Sta. Res. Bull.* **355** *(1942)*) when they devised a satisfactory method for the iodination of proteins, particularly casein. Using a commercial preparation of this type (protamone), the Missouri workers have carried out studies on three successive generations of birds, relating thyroid feeding to egg production, weight maintenance, and mortality (C. W. Turner, M. R. Irwin, and E. P. Reineke, *Poultry Sci.* **24,** *171 (1945)*; C. W. Turner, H. L. Kempster, N. M. Hall, and E. P. Reineke, *Ibid.* **24,** *522 (1945)*). In the first study, four lots of 12 White Leghorn hens were fed a suitable ground laying ration. Lot 1 was fed this ration alone, whereas lots 2, 3, and 4 received 5, 10, and 20 g., respectively, of iodinated casein (thyroxine content = 2.7 per cent) per 100 pounds of the basal ration.

Records maintained throughout one year indicated that all the levels of iodinated casein were well tolerated in so far as weight response and mortality were concerned. The birds lost weight slightly after being placed on the protamone diet, but gained it again during the second month and paralleled the controls thereafter. There was no relationship between dosage and mortality. However, the egg production was markedly affected. Lot 1 (control) birds maintained an average production of 22.6 per cent throughout the year, whereas in lots 2, 3, and 4 the average levels (in per cent) were 38.1, 40.6, and 30.7, respectively. The optimum concentration of protamone was concluded to be between 5 and 10 g. per 100 pounds of feed.

Additional experiments using 10 g. of protamone per 100 pounds of feed (0.02 per cent) showed that the greatest effect of the thyroid feeding was to delay the decline in egg production which accompanies the normal decline in thyroid secretion, beginning in April and continuing until October. Twelve Rhode Island Red hens on the control diet maintained an average production of 70.8 per cent per day from November to April, then declined steadily, reaching 40 per cent on August 6 and continuing to 34 per cent at

the end of the experiment in October. The average for the period from April to October was 52.7 per cent. By contrast, 12 hens on protamone, after reaching peak production two weeks later than the controls, maintained production at 70 per cent or above until August 20, then declined rapidly to the same level as the controls at the end of the experiment. The averages for this group were 71.6 per cent from November to April, and 67.2 per cent from April to October. The yearly yield was 235 eggs per hen in the control group compared with 258 in the protamone group. White Leghorn hens responded similarly, although less dramatically.

The reverse condition, namely, that of hypothyroidism, has also been studied in poultry. Hypothyroidism may be induced either by diets low in iodine (L. W. Nicholson and W. R. Breneman, *Poultry Sci.* **24,** *426 (1945)*), or by feeding goitrogenic substances such as 2-thiouracil or various thioureas which inhibit the secretion of thyroxine (C. G. MacKenzie and J. B. MacKenzie, *Endocrinology* **32,** *185 (1943)*). Thiouracil has been employed by J. P. Mixner, E. P. Reineke, and C. W. Turner (*Ibid.* **34,** *168 (1944)*; H. L. Kempster and C. W. Turner (*Poultry Sci.* **24,** *94 (1945)*; and F. N. Andrews and E. E. Schnetzler (*Ibid.* **25,** *124 (1946)*). Enlarged thyroids resulted on both types of goitrogenic diets. Body weight was not materially affected on the iodine-low diet, and although thyroid hyperplasia was encountered, Nicholson and Breneman concluded that this condition was "not necessarily detrimental to the growth or sexual development of the chick up to eleven weeks of age." On a diet containing 0.2 per cent thiouracil, however, body weights were decreased about 13 per cent in broilers fed from the sixth to the fourteenth week of age. Andrews and Schnetzler report further that the basal metabolic rate was sufficiently reduced to permit approximately a 9 per cent greater deposition of fat in the skin, and an increase in the $\frac{\text{gain}}{\text{feed}}$ ratio from 0.275 in the controls to 0.290 in the birds fed thiouracil. This conclusion does not appear to be completely established, however, for they also observed an increase in body temperature of 0.22° in the thiouracil flock. Further, Kempster and Turner report an increased $\frac{\text{feed}}{\text{gain}}$ requirement with thiouracil. The reason for this discrepancy is not apparent from the publications.

Market quality of the birds as evidenced by fleshing was greatly improved by thiouracil feeding.

Although enormous increases in size (twenty to seventy times) were produced in the thyroid gland by feeding thiouracil, Andrews and Schnetzler reported that no toxic effects were noted upon gross or histopathologic examination of the liver and kidney. Comb area and testis weight were reduced significantly.

CALORIC INTAKES OF A GROUP OF BOSTON CHILDREN

The incompleteness of the present knowledge of energy metabolism has been emphasized and conservatism urged in the application of dietary standards alone as a measure of the adequacy of caloric intake (*Nutrition Reviews* **4,** *34 (1946)*). The value of dietary studies in contributing to an increase in our knowledge of caloric needs was pointed out. Additional data on the caloric intake of children living at home have been presented by V. A. Beal, B. S. Burke, and H. C. Stuart (*Am. J. Dis. Child.* **70,** *214 (1945)*). As a portion of a fifteen year study of growth and development of the well child conducted by the Department of Child and Maternal Health of the Harvard School of Public Health these workers have analyzed

at six month intervals the caloric intake of 324 children aged 1 through 10 years.

These estimates of intake were calculated from one-day dietary records kept by the parent and from nutritional histories taken from the mothers at the clinic. A comparison of the means of the intakes as calculated from the two sources shows rather good agreement between the two methods at all age groups from 1 through 10 years; however, during the first 7½ years the records kept by the mothers tended to be somewhat higher (approximately 50 to 150 calories) than the histories obtained by the nutritionists. At about 8 years the records kept by the mothers tended by a like amount to fall below those calculated from the history. The workers express the belief that the mean of these two estimates is more representative of the true intakes of the subjects.

The estimated mean caloric consumption increased with remarkable regularity by 100 to 200 calories per year for both girls and boys from 1 year of age to 9 years of age. There was essentially no difference between the estimated mean intake at 9 and 10 years for either sex. From the fifth year the mean calculated caloric intake of girls was slightly lower than that of boys. The mean of the estimated intakes rose from 1224 calories at 1 year for both sexes to 2328 calories for boys 10 years of age and 2096 calories for girls 10 years of age. These means are somewhat above the National Research Council's Allowances (*Nutrition Reviews* **3**, *287* (*1945*)) for the age group involved. Furthermore, these means are higher than most of the estimates of intake of similar groups recorded by other investigators. Thus, in a study of the effects of dietary improvement on children 6 to 10 years of age in an institution it was found that the subjects voluntarily restricted their caloric intakes to a level below the recommended allowance (*Nutrition Reviews* **4**, *247* (*1946*)). At the same time these latter subjects were gaining weight at a greater-than-expected rate. I. G. Macy ("Nutrition and Chemical Growth in Childhood," I, *p. 137, Springfield, Ill.* (*1942*)) records the average of determined physiologic fuel values of the diets of 18 children during two thousand, four hundred and forty experimental days and these averages are approximately 100 to 300 calories lower for the ages 5 to 9 years than the intakes recorded by Beal and her co-workers.

The data presented in the present communication do not permit interpretation of this discrepancy, but a number of possible explanations come to mind: (1) Some constant systematic error may have occurred in the estimation of the quantities concerned. (2) This population may be an entirely different type from the groups of children usually studied. It seems inconceivable that continuous contact with a well-child clinic for a period of years would not result in a quality group different from that which one would find in a cross section of the population. (3) It is possible that the mean values are influenced unduly by grossly excessive intakes by a few individuals within each group, i.e. that the mean intakes are greater than the median (the medians are not reported). (4) Inasmuch as the dietary records were kept for but a single day at a time there may have occurred a tendency for the child to overeat on that particular day. If so, such a dietary pattern would be fixed in the mind of the mother and might possibly also influence her estimate of the child's usual intake. Comparison of data obtained from consecutive dietary records for longer periods of time, for example seven days, would be of interest. (5) These investigators may have been dealing with children who were above the usual weight standards. Future reports will assist in clarifying these points.

It is often argued that in dietary studies of this type the large number of intake records tend to cancel out errors in estimates and evaluation and daily variations in intake. This is true only as regards accidental errors, but any systematic bias which may exist in

these estimates will remain unaltered despite the large numbers of estimates.

It is almost impossible to devise a direct measure of the accuracy of such dietary records as those upon which this study is based. Some of the efforts to determine the validity of dietary records have been discussed previously (*Nutrition Reviews* **2,** *184 (1944)*). Until more critical examinations are made by each center employing such records it is well to regard the results of dietary records merely as of relative value, and to refrain from interpretation of the absolute values so obtained in terms of physiologic requirements. In a critique of similar technics for nutrition studies Professor E. J. Bigwood has written: "When the methods adopted produce enough data to conform to strictly statistical requirements, they frequently yield only approximations and are hence not suited to the requirements of the physiologist" ("Guiding Principles for Studies on the Nutrition of Populations," *p. 39, League of Nations Health Organization, Geneva (1939)*). It is well to recognize this principle in interpreting such data.

SUPPLEMENTATION OF THE DIET OF AIRCRAFT WORKERS

Proposed criteria of nutritional deficiencies have often been taken as measures of the prevalence of nutritional deficiencies in the population before adequate tests of their validity have been made (*Nutrition Reviews* **1,** *118, 194 (1943);* **3,** *38 (1945)*). Thus, the prevalence of certain biomicroscopic changes has been interpreted as indicating the prevalence of deficiencies (Committee on Diagnosis and Pathology of Nutritional Deficiencies, Food and Nutrition Board, *Bull. National Research Council no.* **109** *(1943)*), although the validity of these methods had not been subjected to rigid tests.

An adequate test of a proposed criterion of deficiency was summarized by J. R. Marrack ("Food and Planning," *p. 53, Victor Gollancz, Ltd., London (1943)*) as follows: "Before concluding that any symptom is due to deficiency of a particular vitamin, we should insist that the incidence of this symptom should be related to the amount of this vitamin in the patient's diet and that there should be a higher rate of recovery among patients treated with this vitamin than among control cases. The most satisfactory information is that obtained from the few deliberate experiments in which the vitamin supply of human beings has deliberately been limited." Many carefully executed nutrition surveys provide one type of the evidence called for by Marrack—a test of whether a given finding is correlated with dietary intake as estimated from diet records or as reflected by laboratory analyses of blood. Since 1943 several well controlled investigations of the effects of dietary supplements on biomicroscopic lesions have been reported and a few experiments of the effects of restricted diets have appeared. Many of these have been reviewed in this journal.

Additional evidence on the effect of dietary supplementation on biomicroscopic lesions is provided by a fourth report in the series entitled "Nutritional Status of Aircraft Workers in Southern California" (H. Borsook *et al., Milbank Memorial Fund Quart.* **24,** *99 (1946)*). In this study a supplement containing 50,000 I.U. of vitamin A, 800 I.U. of vitamin D, 10 mg. of thiamine, 10 mg. of riboflavin, 100 mg. of nicotinamide, 250 mg. of ascorbic acid, and 500 mg. of calcium as calcium carbonate was administered five days each week to one group of workers (the vitamin group). A placebo was administered to a second comparable group (placebo group) and a third control, nonparticipating group, was selected at the conclusion of the experiment. The general plan of the experiment and the dietary intakes, initial findings on physical examination, and the effects of the supplementation program

on absenteeism, personnel rating, and turnover have been reviewed (*Nutrition Reviews* **1,** *238 (1943);* **2,** *107 (1944);* **3,** *297 (1945)*). The present report compares the clinical and laboratory findings of the groups with each other at the conclusion of a year of supplementation as well as each group before and after supplementation.

Unfortunately, different criteria and examiners were employed at the two periods of time so that the comparison of clinical findings before and after supplementation has little value except to illustrate that the incidence of a given minor physical finding is more dependent upon the judgment of the examiner than upon the nutritional state of the individual. For example, 6.2 per cent of the persons in the vitamin group were judged initially to present one or more skin signs "attributed to vitamin A deficiency." By contrast 73.7 per cent of the vitamin group presented skin signs "attributed to vitamin A deficiency" after supplementation for one year with a total dosage of 13,000,000 I.U. of vitamin A from fish liver oil (or sufficient for seven years allowance). To one familiar with the pitfalls of the subjective grading of the differences in skin texture encountered in the population, it is apparent that such gradings are most unsatisfactory. It seems unfortunate that the criteria were changed from the more objective findings used during the first examination period to the difficultly definable gradings of "whether or not the hair seemed abnormally dry, and any degree, no matter how slight, of follicular hyperkeratosis." Similar examples of the great variations between subjective gradings are to be found in the sections of this paper dealing with signs resembling those seen in vitamin B-complex deficiencies on the lips and face.

Comparisons of the placebo with the vitamin group on the second examination are more valid. The most interesting of these comparisons may be summarized briefly:

(1) The ratings of thickness of the conjunctiva as judged by examination with the slit-lamp revealed no statistically significant differences between the treated and untreated groups. There was some slight difference in ratings in certain areas of the conjunctiva in favor of the supplemented group, but it was noted that the same individual might have "higher ratings for several areas and lower ratings for other areas" on two consecutive examinations. The authors state "The superiority in the change for the vitamin subjects is less than might be expected from Kruse's report." No reduction in the elevation of the conjunctival spots was found in the vitamin subjects.

(2) Detailed ratings of corneal vascularization, so frequently used to assess "riboflavin deficiency," revealed no differences between the placebo and vitamin groups either before or after supplementation. The authors state "We must conclude that corneal vascularity, even as extensive as that seen in a large number of our subjects, may not be taken as evidence of existing riboflavin deficiency." They express the reasonable conviction that a longer period of therapy would not have been more effective.

(3) The data on abnormalities of reflexes, calf tenderness, plantar dysesthesia, and loss of vibratory sense to C-256 tuning fork revealed no differences between the placebo and vitamin groups. Quantitative measures of the thresholds of vibratory sense (G. Keighley, *Milbank Memorial Fund Quart.* **24,** *29 (1946)*) revealed no differences between the two groups. One cannot attribute the observed abnormalities to deficiencies of thiamine, riboflavin, or niacin.

(4) Changes in the lips, nasolabial folds, or face attributed to vitamin B-complex deficiencies did not differ in the two groups after a year of supplementation.

(5) The skin texture of the vitamin group was graded as somewhat different from that of the placebo group. This slight difference occurred in four of the ten criteria adopted in a statistically significant magnitude. These criteria, the authors note, were not well defined. Furthermore, they are criteria which are so related that they constitute but one

attribute, i.e. dryness. It is not surprising that "dry skin" and "dry hair" would be found to be associated. The authors conclude that the data suggest a possible effect of the supplementation on skin and hair texture and "warrant adequately controlled therapeutic tests of the suggestion they offer." No claim is made that they constitute an adequate criterion of any deficiency state to be employed in the general population.

(6) Results of the second examination indicated slight differences in changes of the tongue attributed to vitamin B-complex deficiency. These differences were in favor of the supplemented group, but the authors emphasized that they consider them suggestive only.

(7) The mean plasma ascorbic acid rose in the vitamin group, but no data were compiled for gingival changes. It is of interest that on such a huge ascorbic acid intake as 250 mg. five times a week that 7 of the 264 members of the vitamin group had plasma ascorbic acid levels less than 1.0 mg.

(8) The hemoglobin values of the persons with less than 14.0 g. on the first examination were unchanged by the supplementation.

(9) No therapeutic effect of the supplementation could be ascertained on a large variety of symptoms relating to the eyes, gastrointestinal tract, fatigability, and appetite. The control group which had received neither vitamin nor placebo reported fewer symptoms than did the study group. This fact indicates that some selection existed in the sampling.

(10) Over 70 per cent of the placebo group reported distinct benefits from the pills which they had been taking. The psychologic benefit of participation in an experiment is well illustrated by this finding.

These well presented results merit many comments. Taken together with the similar negative results of other investigators who have attempted to reverse the common conjunctival changes by prolonged administration of vitamin A or by liberal dietary intakes of vitamin A (E. C. Robertson and A. L. Morgan, *J. Nutrition* **31,** *471 (1946);* J. Calvo *et al., J. Am. Dietet. Assn.* **22,** *297 (1946)*) and the epidemiologic data indicating a total lack of correlation between dietary intake or blood vitamin A levels and these findings in the population (R. K. Anderson and D. F. Milam, *J. Nutrition* **30,** *11 (1945);* W. J. Darby and D. F. Milam, *Am. J. Pub. Health* **35,** *1014 (1945)*) it can be concluded that such biomicroscopic changes are of no value in assessing the vitamin A status of a population. Similarly, the failure of the commonly occurring corneal vascularity to disappear after treatment with riboflavin, and the epidemiologic studies demonstrating a lack of correlation between the intake of riboflavin and the presence of this sign (J. B. Youmans, E. W. Patton, W. D. Robinson, and R. Kern, *Tr. Assn. Am. Physicians* **57,** *49 (1942)*; R. K. Anderson and D. F. Milam, *J. Nutrition* **30,** *17 (1945)*; W. J. Darby and D. F. Milam, *loc. cit.*) demonstrate that this sign alone cannot be taken as a measure of riboflavin deficiency in a population. Similar conclusions can be stated for neurologic findings and thiamine deficiency, as well as medical history and vitamin B-complex deficiencies. Reinterpretation of much of the earlier data on the incidence of deficiency states as judged by these and similar criteria seems to be in order. Most of the tests which have been applied are not specific and do not measure dietary deficiencies. Finally, despite the fact that these aircraft workers had dietaries which did not approach the ideal as usually depicted, this and the preceding reports do not reveal any impressive benefits to be derived by the routine provision of large vitamin supplements to industrial workers whose food habits are comparable to these. Such findings force the realization that there is still much information to be gained concerning the significance for health of the various zones of nutriture which exist in the American population.

RELATION OF THE KWASHIORKOR SYNDROME TO ARIBOFLAVINOSIS

For some years there have appeared reports from Kenya, the Cameroons, and the Gold Coast of a form of severe malnutrition in native children variously termed "Gillan's edema," "cheveux blancs," "enfants rouges," "infantile pellagra," "Kwashiorkor" (Kwashi: name of a boy born on Sunday; orkor: red), or "malignant malnutrition." This disease, as reviewed by H. C. Trowell and E. M. K. Muwazi (*Arch. Dis. Childhood* **20**, *110* (*1945*)), is characterized by failure of growth, edema, macrocytic anemia, steatorrhea, a characteristic dermatosis, potbelly, fatty liver, and decreased plasma albumin but increased plasma globulin, and a deficiency bowel pattern on x-ray examination. Some 50 per cent of the cases occur in children under 2 years of age. Trowell and Muwazi estimate that about three quarters of the sick children in Kampala, Uganda suffer from varying degrees of this condition. The disease occurs less frequently in the adult. No cases have been reported among European, white American, Chinese, or Japanese children.

The skin lesions of Kwashiorkor have attracted considerable attention because they result in depigmentation of both skin and hair, and in alopecia. The pallor and redness of the skin and hair are reflected in the name of the disease. These changes are well depicted by W. Hughes (*Tr. Roy. Soc. Trop. Med. Hyg.* **39**, *437* (*1946*)). Trowell and Muwazi note that the skin lesions are not typical of pellagra and that niacin is ineffective therapeutically.

The type of anemia is stated by Trowell and Muwazi to be macrocytic but hypochromic and the bone marrow to be devoid of megaloblasts, the typical cells of pernicious anemia, sprue, and nutritional macrocytic anemia. Infections are common; malaria was encountered in two thirds of the cases, intestinal parasites in one third, and pneumonia commonly. Sickle-cells were present in one fifth of the cases. The influence of these other diseases on the development of the syndrome cannot be defined. The distinction between celiac disease, cystic fibrosis of the pancreas, and Kwashiorkor has not been carefully analyzed.

Trowell and Muwazi did not study the liver involvement in this disease (T. Gillman and J. Gillman, *J. Am. Med. Assn.* **129**, *12* (*1945*)). In a study of liver biopsy material from 20 children suffering from this syndrome, Gillman and Gillman found that all showed various degrees of fatty changes and that these changes were accentuated in 7 cases treated with a mixture of 5 mg. of riboflavin twice a day and 50 mg. of nicotinamide. All of these patients died. This high mortality justifying the term "malignant malnutrition," is to be contrasted with the American experience with pellagra in childhood as summarized by K. Dodd (in S. Harris, "Clinical Pellagra," *p. 341, C. V. Mosby Co., St. Louis* (*1941*)) to the effect that "The addition of brewers' yeast in 2 to 4 gm. doses three times a day will hasten the cure in most cases. . . . Nicotinic acid in 10 mg. doses for infants and 25 mg. doses for older children three or more times a day now offers a very easy method of obtaining a cure." This difference in response further differentiates Kwashiorkor from pellagra.

The successful use of powdered stomach introduced by Gillman and Gillman in the treatment of this syndrome has been reviewed (*Nutrition Reviews* **4**, *52* (*1946*)). Trowell and Muwazi state that their experience in 6 cases with this agent is not conclusive but that the results are encouraging.

A good picture of Kwashiorkor as observed in Nigeria is provided by the report of W. Hughes (*loc. cit.*). Hughes emphasizes the lethargy and preterminal coma with abrupt unexpected death and the fatty infiltration of the liver as seen on autopsy. He stresses the similarity of these two findings to the sudden coma and death and the yellow (fatty) livers found by W. H. Sebrell and R. H. Onstott (*Pub. Health Reports* **53**, *83*

(1938)) in dogs deprived of riboflavin. The possible relation of the liver damage to methionine deficiency is also mentioned.

These considerations, taken together with the similarity of the skin lesions in this disease to the ariboflavinotic lesions and the occurrence of Kwashiorkor in a population with a background of ariboflavinosis in 16 per cent of the adult group, led Hughes to test the efficacy of riboflavin in the treatment of the syndrome. One case which recovered after therapy with 12 mg. of riboflavin alone is reported. Seven other cases treated with this agent in smaller amounts died a few days after admission despite some clearing of the skin lesions.

The usual dietary of these children is composed of bulky carbohydrate of vegetable origin: plantains, cassava tubers, sweet potatoes, millet, maize, and ground-nuts. Little meat, milk, or other good sources of animal protein are taken. The infants may be weaned to a diet of nothing but "pap," a gruel made fom guinea-corn. Such a diet is bulky, low in protein, and probably also in riboflavin.

Hughes' concept of the syndrome appears to be that Kwashiorkor is a complex deficiency state in which ariboflavinosis is an outstanding feature. The resulting liver damage may be so severe that adequate curative measures are difficult. Hence, the prevention of the disease is the solution to its refractory nature. He suggests that food yeast (*Nutrition Reviews* **3,** *286 (1945)*) should make prevention practical. The reasonableness of Hughes' concept would appear to make a properly designed test of the hypothesis highly desirable. Certainly his reported therapeutic efforts are not conclusive.

The prevalence among natives of this syndrome of malnutrition involving such extensive liver damage brings to mind the very high incidence of primary hepatic malignancies among African natives (*Nutrition Reviews* **3,** *19 (1945)*).

PELLAGRA

Although reasonably exact dietary records on somewhat less than 2000 human beings seem meager in contrast to the thousands of experimental animals on known diets under controlled conditions, the studies of E. I. Frazier and T. E. Friedemann (*Quart. Bull. Northwestern Univ. Med. School* **20,** *24 (1946)*) on human pellagra are nevertheless outstanding. These workers emphasize that "the history of pellagra is of interest to the research worker because it shows how slowly the science of nutrition advances without experimentation," and "the study of pellagra, as carried out by Goldberger and others since, is of particular interest to the investigator because it represents the successful application of three important methods to the study of human nutrition: survey, clinical observation and experiment." In the introduction to their review they discuss the contribution of T. Roussel to the subject of nutrition in his classic "Traité de la Pellagre," *Paris (1866)*. It is not likely, however, that the modern view of pellagra was first stated by him. Indeed it is almost impossible to assign to any single student of the disease the major credit for emphasizing the primary role of malnutrition in its etiology. Perhaps the first demonstration of the importance of proper food in prevention and treatment of pellagra was an experiment done by Giuseppe Cerri in 1795. Ten persons with typical pellagra who were provided with good food for a period of more than a year all had rapid remission of the disease and none had an attack during the succeeding spring. Others whose diets were not changed had regular seasonal relapses.

Frazier and Friedemann present an elaborate analysis of the studies of human

pellagra during the epoch inaugurated by J. Goldberger (*J. Am. Med. Assn.* **66,** *471 (1916)*). Tables of dietary intake from several workers were examined for protein, fat, carbohydrate, thiamine, riboflavin, and niacin and the daily nutrient intake was determined. A total of 1813 subjects had records adequate for analysis. Although some of the dietary data were only semiquantitative the large number of cases and the criterion of advanced pellagra (dermatitis, glossitis, diarrhea, and often dementia) enhanced the value of the analysis. From a study of each of the experimental diets several interrelated factors appeared to be involved in the occurrence of signs and symptoms of niacin deficiency in man on a diet containing corn. These were (1) the quantity of animal protein, (2) the quantity of niacin, (3) the interrelationship of various fractions of the vitamin B-complex, especially niacin and riboflavin, and (4) the general composition of the diet.

J. Goldberger and G. A. Wheeler (*Pub. Health Reports* **30,** *3336 (1915)*) in their early work noted specifically that the amino acid mixture was deficient in the dietary of pellagrins. In view of the recent demonstration of the sparing action of tryptophane on niacin requirements in rats and dogs fed a diet containing large amounts of corn (*Nutrition Reviews* **3,** *267 (1945)*), it is of particular interest that J. Goldberger and W. F. Tanner (*Pub. Health Reports* **37,** *462 (1922)*) noted improvement in pellagrins given cystine and tryptophane. The beneficial effect of tryptophane when added to a diet containing the corn protein zein had been known since the work of E. G. Willcock and F. G. Hopkins (*J. Physiol.* **35,** *88 (1906)*).

Data presented by Frazier and Friedemann indicate that the same relationship between protein and niacin holds true in human nutrition as has been demonstrated in animals. In some instances the difference in niacin content between a pellagra-preventing and a pellagra-producing diet was insignificantly small. It appeared that where the content of good protein or riboflavin or both was high a relatively low content of niacin did not result in pellagra. Many of the high vegetable diets prevented pellagra by virtue of something other than the content of niacin. When the diet contained much corn the level of niacin necessary to prevent pellagra was found to be somewhat higher than in a similar diet with little or no corn. This evidence that the minimum requirement for niacin in man is affected by the general composition of the diet is of considerable importance since the accepted method for establishing requirements of vitamins assumes that if adequate levels of all save the tested factor are maintained the prevention or cure of symptoms will occur when the test vitamin is provided in sufficient quantities.

Some of the other conditions which have seldom been controlled or evaluated qualitatively in experiments on human nutrition include metabolism, both basal and total, alimentary synthesis, availability of preformed or synthesized factors, intestinal motility, vitamin storage, environmental temperature, genetic and racial factors, and sex. Perhaps some of these may be responsible for the enigma of pellagra discussed by R. E. Remington (*South. Med. J.* **37,** *605 (1944)*) who has focused attention on the difficulty of interpreting morbidity and mortality statistics in terms of present knowledge of deficiency diseases. W. DeKleine (*Ibid.* **35,** *992 (1942)*) noting the decrease in mortality from pellagra in the southern states from 22.4 per 100,000 in 1928 to 5.1 per 100,000 in 1940, attributed it to the use of brewers' yeast, promotion of gardening, introduction of niacin, and to education. Remington found that the data were not in accord with the conclusions reached by DeKleine. In an effort to relate the mortality from pellagra to the economic status of the population, figures for mortality have been compared with the cotton index for South Carolina. There was no indication that pellagra had increased after the income or buying power decreased, nor was there any

relation, because the worst years of the depression witnessed a 77 per cent reduction in mortality among Negroes and a 40 per cent reduction among whites. There was no significant difference in mortality in rural and urban populations. Mortality was high among females during the age of childbearing and at the menopause. No objective study has shown that pregnancy and lactation are responsible, though they are the most probable etiologic factors.

The possible effect of improved diets and brewers' yeast were investigated by comparing the mortality and morbidity figures with those for the quantity of yeast distributed by the State Board of Health from 1928 through 1943. The decrease in mortality did not appear to result from the recovery of a greater number but from the fact that fewer contracted the disease. The decrease in bulk distribution of yeast followed the decline in morbidity and mortality, indicating that yeast was used as a remedy rather than as a prophylactic agent. Plotting the logarithms of incidence and death rates against time, straight lines could be drawn for the years 1929 to 1935 and for 1938 to 1942 inclusive, with a distinct break for 1935 to 1937. Incidence and mortality rates decreased at the same rate in both periods. The decrease in the ratio of deaths to incidence after 1938 may be taken to indicate that the remedial measures since that time have been more effective and it is possible that the niacin has been responsible for this change.

Is the sixfold or sevenfold reduction of incidence of pellagra from 1929 to 1942 an indication of a widespread improvement of some or all of the essential components of a good diet? Is the reduction of mortality and morbidity from pellagra by half from the years 1936 and 1937 (when Stiebeling reported that 40 per cent of the people of the southern states received inadequate nutrition) to the years 1941 and 1942, a sign of a similar decrease in general malnutrition? These questions can be answered by studies such as those carried out by Frazier and Friedemann where quantitative diets can be evaluated from more than one standpoint.

METHIONINE INTAKE AND THE RATE OF TRANSMETHYLATION

M. Cohn, S. Simmonds, J. P. Chandler, and V. du Vigneaud (*J. Biol. Chem.* **162,** *343 (1946)*) have described an investigation into the effect of the methionine intake of the rat upon the rate of transmethylation reactions within the body. The method first tried was to feed methionine with labeled hydrogen in the methyl groups at two dietary levels for one week, and then to kill the animals and isolate their tissue choline and creatine for determination of the deuterium content.

For this experiment two pairs of young rats, each weighing about 65 g. were chosen, and for the seven days of experimental feeding 2 of them received a simplified diet containing 0.7 per cent deuteriomethionine and the other 2 a similar diet containing 1.4 per cent deuteriomethionine. The rats of the two groups were pair-fed. At the end of this time the choline and creatine of the tissues were isolated as choline chloroplatinate and creatinine potassium picrate respectively. Analysis of these compounds showed that the deuterium content of the specimens isolated from rats fed 1.4 per cent was more than 50 per cent higher than in those from rats which had received 0.7 per cent deuteriomethionine. The variability of the figures in each group of 2 is sufficiently large to suggest the real need of using larger groups of rats.

Interpretation of this result is difficult. If the dietary level of deuteriomethionine had no effect on the amount of deuterium in the isolated choline and creatine, it would have led to the conclusion that the dietary level does not affect the rate of transmethylation; the opposite finding, which appeared from their experiment, does not justify the opposite conclusion. The difference in the amount of deuterium in the choline and creatine at the different dietary levels of deuteriomethionine might have been due either to different rates of transmethylation, or to different concentrations of the isotopic methionine participating in the transmethylation. Lack of suitable quantitative methods prevented determination of the actual deuteriomethyl content of the methionine of the tissues, so that another type of experiment was devised to distinguish between the alternative explanations of the experimental result outlined above.

Five adult rats were fed deuteriocreatine in addition to their normal casein diet for twenty-one days and then deuteriocreatine was discontinued and the rats separated into two groups. One group was fed a diet containing casein sufficient to supply 0.7 per cent methionine, while the second group received the same diet supplemented with dl methionine to raise the total concentration of the amino acid in the diet to 1.4 per cent. During the next forty days creatinine was isolated at intervals from the urine excreted by each group and its deuterium content was determined. Since it had been shown by V. du Vigneaud and coworkers (*J. Biol. Chem.* **140,** *625 (1941)*) that on a creatine-creatinine-free diet the deuteriocreatinine excreted reflects the isotope content of the tissue creatine, the observed changes in isotope content of the creatinine were here used to follow the changes in tissue creatine throughout the forty days. At the end of the period the tissue creatine was isolated and analyzed as a check on the urine measurements, which it corroborated closely.

Because it is known that the total amount of creatine in the animal body and its excretion remain constant, and that the nitrogen of creatine is not used in anabolic reactions, the rate of disappearance of isotopic creatine must be equal to the rate of synthesis of new creatine in the body. Different circumstances surrounding the methylation of ethanolamine to form choline make it impossible at present to measure indirectly the rate of formation of choline in a way similar to that used for measuring the formation of creatine.

The measurement of the rate of formation of creatine in the two groups of rats receiving different levels of methionine did not show any difference between the groups, whereas if the rate of formation were affected by the level of the precursor in the diet, the diet containing the higher level of methionine should have produced the faster synthesis of creatine. Since this did not occur, the authors concluded that the dietary level of methionine does not affect the rate of transmethylation.

Similar measurements on the rate of disappearance of isotopic creatinine from the urine after a period of feeding with isotopic creatine were made by K. Bloch, R. Schoenheimer, and D. Rittenberg (*J. Biol. Chem.* **138,** *155 (1941)*). They used creatine containing N^{15} instead of deuterium. The half-life of the N^{15} creatine was found by them to be twenty-nine days, whereas the new data with deuteriocreatine give a figure of approximately thirty-six days. This agreement is perhaps as close as could be expected when such small groups of animals were used, but to determine whether a real difference exists between the two types of isotopic creatine appears to demand the use of considerably more animals.

TOOTH DECAY IN THE COTTON RAT

Studies on the relation of purified diets to tooth decay in the cotton rat have been reviewed previously (*Nutrition Reviews* **3,** *83 (1945)*; **4,** *73 (1946)*). A high incidence of tooth decay was observed in the molar teeth of cotton rats maintained on diets high in sucrose or other soluble carbohydrates. A much lower incidence of carious lesions was observed when high levels of fluorine were added to the sucrose basal ration, or when dextrin replaced the sucrose in the basal ration completely or when rations composed of natural foodstuffs were fed.

In a recent paper B. S. Schweigert, J. H. Shaw, M. Zepplin, and C. A. Elvehjem (*J. Nutrition* **31,** *439 (1946)*) have described a series of experiments in which cotton rats were fed whole milk diets and diets in which varying amounts of sucrose were replaced by fat, protein, or a combination. Complete prevention of tooth decay was observed when weanling cotton rats were fed whole milk supplemented with ample iron, copper, and manganese salts as the sole source of nutrients. When a diet approximating the composition of milk solids (salts 5.4 per cent, casein 27.3 per cent, lactose 39 per cent, and butterfat 28.3 per cent) was fed to cotton rats, a very low incidence of carious lesions developed in comparison to the high incidence in the litter mates maintained on the sucrose basal ration.

The substitution of lard for sucrose was made isocalorically in the experiments on the relation of dietary fat content to the incidence of carious lesions. In this way the ratio of caloric intake to protein, mineral, and vitamin consumption was maintained. When 45 parts of the sucrose in the basal ration were replaced by 20 parts of lard, there was a very low incidence of carious lesions in the cotton rats to which this high fat diet was fed. When 22.5 parts of sucrose were replaced by 10 parts of lard, the incidence of carious lesions observed was about half way between the incidence observed in the control animals fed the sucrose basal ration and those fed the ration with the higher fat content.

When the protein content of the diet was increased from 24 to 50 per cent by the replacement of 26 parts of the sucrose with casein, a lower caries incidence occurred in the rats fed this ration than in their litter mates which were fed the sucrose basal ration. The reduction in caries incidence observed in this case was similar to that observed in those cotton rats which received the diet with the medium fat content. When a diet was fed which contained the same amount of lard as the medium fat diet and the same amount of casein as the high protein diet, the incidence of carious lesions was as low as that observed when the higher fat ration and the milk solids ration were fed.

These experiments indicate that a variety of different dietary manipulations can be made which will alter the incidence of tooth decay in cotton rats. Possibly the most interesting observation that has been recorded about the effect of diet upon tooth decay in the cotton rat was the complete prevention of carious lesions observed when a diet consisting solely of mineralized milk was fed. Yet a diet composed of mineral salts, casein, lactose, and butterfat in the proportion present in cow's milk did not give as good protection. Possibly the composition of the minerals in milk or the fluidity of the ration may have contributed to this difference.

Studies with the cotton rat on the relation of purified diets to tooth decay have yielded some interesting results of a type not possible previously in experimental animals. As yet no minute description of the carious lesions has been reported in the literature. A thorough histologic study of the initiation and development of these lesions would be an important contribution to the prob-

lem. In this way the relation of plaque and materia alba formation to the initiation of the early stages of enamel destruction and to the further progress of the lesions could be studied effectively. The comparison of such studies with similar investigations of carious lesions in human teeth might indicate some of the similarities and dissimilarities between experimentally produced lesions and the naturally occuring ones in man.

REGENERATION OF LIVER PROTEIN AS A TEST FOR DIETARY PROTEIN ADEQUACY

It has long been known that during short periods of fasting or feeding on diets low in protein, the liver of the rat decreases in weight rapidly, and that part of this weight decrease is due to loss of protein. No other tissue is known to lose protein as rapidly as the liver under such conditions. The work of H. W. Kosterlitz (*Nature* **154,** *207* (*1944*)) indicated that the protein lost is actually liver cytoplasma, and that the liver nitrogen, expressed in terms of body weight, is a function of both the amount of protein and the type of protein fed. He also revived an earlier suggestion of T. Addis, L. J. Poo, and W. Lew (*J. Biol. Chem.* **116,** *343* (*1936*)) that the rate of regeneration of liver protein following renewed feeding with protein might be used as a basis for the assay of the nutritional adequacy of proteins. He suggested that the changes in cytoplasmic substance, which may be closely estimated by measuring the nonglycogen nonlipid solids of the liver, might be used for the rapid measurement of the biologic value of the proteins.

H. C. Harrison and C. N. H. Long (*J. Biol. Chem.* **161,** *545* (*1945*)) have followed up this line of inquiry with positive results. Using male rats for study, these animals were kept for at least a week on a simplified stock diet containing casein, corn starch, Crisco, cod liver oil, yeast, and salt mixture. Rats weighing 250 to 280 g. were fasted for forty-eight hours, and then fed a simplified diet containing the protein to be tested. Twelve g. of the test diet were fed on the first day of refeeding, and 10 g. on each of the next three days. The only variable among the test diets was the protein used; all the diets were isocaloric with the stock diet, and each contained 3.9 per cent of yeast protein supplied by the 8 per cent of yeast it contained. At the end of the four-day refeeding period the rats were anesthetized with nembutal and exsanguinated from the abdominal aorta, and the livers removed and dried to constant weight at 95°. The dried liver was ground to a fine powder and aliquots for estimating total nitrogen and total lipids were taken.

Preliminary experiments were made to determine the optimum length of the refeeding period. After a forty-eight hour fast, compared with unfasted controls, the rats lost 32 per cent of the liver nitrogen, and the loss of body weight was about 24 g. After four days of refeeding the 20 per cent casein stock diet, the rats regained their initial body weight, and the liver nitrogen reached a plateau in three to four days. Four days was therefore chosen as the optimum refeeding period. Lactalbumin behaved almost identically with casein, and feeding at a 10 per cent level in the diet on the same caloric intake did not produce any significant increase in the liver nitrogen as did the 20 per cent protein diet.

Gelatin was tested in order to observe the response to a known nutritionally inadequate protein, and it produced no gain in body weight and no regeneration of liver protein. Zein and gliadin were also tried at a 40 per cent level in the diet, and found to produce liver nitrogen regeneration, but significantly

less than similar quantities of casein or lactalbumin.

The effect of a combination of casein and certain amino acids providing nitrogen equivalent to 10 per cent of casein was investigated. Eight per cent casein plus 2 per cent methionine produced a significant regeneration of liver nitrogen in four days, but 8 per cent casein plus 1.2 per cent alanine (containing an equal amount of alpha-amino nitrogen) gave no regeneration, while with 2 per cent of cystine or homocystine the result was intermediate between those with alanine and with methionine respectively.

The interrelationship between water content of the liver and other constituents was examined, and the effect of glycogen was found to be most important in determining liver weight due to the retention of water along with glycogen. A very high correlation coefficient (r = 0.98) was observed between protein plus glycogen content and water content of the livers, and a smaller coefficient (r = 0.78) between protein content and water content.

The authors consider that their data establish the measure of liver nitrogen regeneration after fasting as a method of assay of the adequacy of dietary protein. The method is simple and rapid, and sufficiently precise that only small groups of rats are needed. The possible improvement of technic by substituting synthetic B-vitamins for the yeast in the diet is mentioned, but evidence from their results suggests that little advantage would be gained by such a substitution.

A preliminary communication has been published by H. W. Kosterlitz and R. M. Campbell (*Nature* **157,** *628* (*1946*)) reporting essentially similar results which they have obtained independently. These authors also noted the importance of the quantity of food consumed in regulating the amount of labile liver cytoplasm (nonglycogen nonlipid solids). Unless at least 26 to 27 calories per 100 g. body weight were consumed daily, the maximal effect of the dietary protein on liver protein or cytoplasm was not obtained. If the caloric intake was reduced 50 per cent below the level indicated during a test of casein, its biologic value appeared to be no higher than that of zein.

H. W. Kosterlitz had previously reported his technic for making the tests (*Nature* **154,** *207* (*1944*)) and in the more recent report found that it can be satisfactorily applied to the assay of protein hydrolysates prepared for therapeutic purposes, to the evaluation of the amount of acid-hydrolyzed casein, and to the determination of the biologic values of flours of different degrees of extraction.

BIOSYNTHESIS OF THE VITAMIN B-COMPLEX AND HUMAN NUTRITION

The isolation and characterization of new vitamins and the demonstration of the existence in tissue extracts of unidentified substances of possible nutritional importance have far outstripped the understanding of the biochemical significance of these nutrients. The original investigations that led to the discovery of vitamins were designed primarily to disclose the nutritive value of milk (F. G. Hopkins, *J. Physiol.* **44,** *425* (*1912*)), inorganic salts (E. V. McCollum and M. Davis, *J. Biol. Chem.* **15,** *167* (*1913*)), and proteins (T. B. Osborne and L. B. Mendel, *Ibid.* **15,** *311* (*1913*)) or the cause of historically important diseases such as scurvy (A. Holst and T. Frölich, *J. Hygiene* **7,** *634* (*1907*)) and beriberi (C. Funk, *J. Physiol.* **43,** *395* (*1911*)). The momentum of the research which successfully related thiamine, niacin, ascorbic acid, and vitamins A and D to human deficiency diseases has produced riboflavin, pantothenic acid, paraaminobenzoic acid, pyridoxine, folic acid, and many more chemical compounds, each

indispensable for the normal nutrition of at least one species. This has been the natural result of intensified interest on the part of increasing numbers of experienced scientific workers, of the use of highly purified experimental food mixtures, and of the use of a greater variety of test species subjected to widely varying conditions. How many of these more recently recognized nutrients will be shown to be important in human nutrition remains to be determined. It is noteworthy that riboflavin was isolated, identified, and synthesized before it was related to a human deficiency syndrome by W. H. Sebrell and R. E. Butler (*Pub. Health Reports* **53,** *2282* (*1938*)), and this may also be true of folic acid (*Nutrition Reviews* **4,** *11* (*1946*)). The contrast between the isolation of thiamine from impure extracts of known effectiveness in the disease, beriberi, and the recognition of the dietary causation of the disease, ariboflavinosis, after the isolation of riboflavin is a pertinent illustration of a new phase in the developing science of human nutrition.

Characteristic of this new phase is the realization that faulty nutrition may be a contributing cause of many human ailments, other than recognized nutritional deficiency diseases, and that certain undetermined circumstances may "condition" an individual in such a manner that nutrients which are ordinarily dispensable, readily obtained in adequate amounts, or required in traces only, become critically important in so far as physical well-being is concerned. Pernicious anemia and other macrocytic anemias are possibly in the category of "conditioned" deficiency diseases. Some cases of pellagra may belong to this group, judging from the observation that the addition of corn to a ration markedly increases the requirement of niacin or tryptophane in rats (W. A. Krehl, L. J. Teply, P. S. Sarma, and C. A. Elvehjem, *Science* **101,** *489* (*1945*)). Instances of "conditioned" nutritional disease are common in animal experimentation and may occur more frequently in man than is generally realized. The origin of these "conditioned" diseases is frequently ill-defined but chronic and acute infections, disturbed metabolism due to lack or imbalance of hormones, and malnutrition due to deficiency or unfavorable proportions of nutrients are some of the probable causes. G. R. Minot (*Ann. Int. Med.* **12,** *429* (*1938*)) has long emphasized the importance of various diseases "conditioning" poor nutrition.

The type and activity of microorganisms inhabiting the alimentary tract, especially the large intestine, definitely influence animal nutrition (*Nutrition Reviews* **1,** *4* (*1942*); **4,** *76*, *116* (*1946*)), profoundly affect studies of vitamin balance in man (C. W. Denko and co-workers, *Arch. Biochem.* **10,** *33* (*1946*)), and may be of considerable importance in human nutrition (*Nutrition Reviews* **3,** *48* (*1945*)). If the synthesis of vitamins by organisms in the intestine is a normal and necessary method of supplementation of the diet, the inhibition of bacterial activity or a change in the type of the intestinal flora might constitute a "conditioning" factor which would predispose man to nutritional disease. The beneficial effects of refection (L. S. Fridericia *et al.*, *J. Hygiene* **27,** *70* (*1927*)), and of coprophagy (*Nutrition Reviews* **1,** *220* (*1943*); **4,** *116* (*1946*)) and the deleterious effect of administration of "sulfa" drugs (*Ibid.* **1,** *35*, *175* (*1943*)) in rats are well established. In man, a dietary deficiency of thiamine or of riboflavin may be masked, in part at least, by what appears to be a production of these vitamins by the intestinal flora (V. A. Najjar and L. E. Holt, *J. Am. Med. Assn.* **123,** *683* (*1943*); V. A. Najjar and co-workers, *Ibid.* **126,** *357* (*1944*)). The effectiveness of folic acid in certain macrocytic anemias is intriguing in this connection because the fecal excretion in normal individuals on an average diet has been reported to be considerably greater than the intake (Denko *et al.*, *loc. cit.*). If part

of the folic acid synthesized by intestinal bacteria is actually available, the appearance or absence of deficiency symptoms on diets low in this vitamin might be determined largely by the intestinal flora. It is conceivable that these bacteria and yeasts may destroy as well as produce vitamins.

The extent of the possible synthesis of nutrients in the alimentary tract is emphasized in the paper by Denko *et al.* (*loc. cit.*), in which the intake and excretion of B-vitamins in 7 healthy young men on a normal diet during a twelve week period are reported. The dietary intake of thiamine, riboflavin, pyridoxine, and niacin exceeded the combined urinary and fecal output. The reverse was true with the other B-vitamins, for which the ratio of excretion to intake was as follows: biotin, 3.8:1; pantothenic acid, 1.1:1; para-aminobenzoic acid, 2.3:1; and, folic acid, 5.5:1. The fecal was greater than the urinary excretion for all of the vitamins except pantothenic acid and pyridoxine. The authors point out that the intestinal production of niacin and of pyridoxine may have been appreciably greater than estimated because neither N′-methylnicotinamide nor 4-pyridoxic acid was determined. Both the niacin derivative (B. C. Johnson, T. S. Hamilton, and H. H. Mitchell, *J. Biol. Chem.* **159,** *231* (*1945*)) and the pyridoxine derivative (*Ibid.* **158,** *619* (*1945*)) occur in the urine in significant amounts following the administration of the respective vitamins. No parallelism between the urinary and the fecal output was noted in any of the test subjects.

The possibility of synthesis of vitamins by intestinal microorganisms in man cannot be ignored in the light of these findings which confirm and greatly extend the earlier observations of others. The excretion of biotin, for instance, in 4 patients on a normal diet was found to be from three to six times the intake (T. W. Oppel, *Am. J. Med. Sci.* **204,** *856* (*1942*)). J. Gardner, A. L. Neal, W. H. Peterson, and H. T. Parsons (*J. Am. Dietet. Assn.* **19,** *683* (*1943*)) have reported that the output of biotin, pantothenic acid, and riboflavin was 107, 93, and 55 per cent of the respective intakes in 3 women on a diet of mineralized milk. In all of these studies, fecal excretion of biotin was greater than urinary excretion. Despite the evidence of bacterial synthesis presented in these balance studies, it would be premature to conclude that synthesis really occurs in each instance and that it protects individuals from deficiencies of biotin, folic acid, and some other B-vitamins, regardless of the dietary intake of these factors. It is possible that natural foodstuffs contain unrecognized combined forms of nutrients which are not detected by present assay methods prior to exposure to the enzymes and microorganisms of the alimentary canal.

If synthesis is accepted as a fact, the question of availability becomes a matter of prime importance. It may be that fecal vitamins of bacterial origin are largely nonutilizable and are, therefore, without significance in balance studies. This possibility is not supported by the observation that 60 to 100 per cent of each of the eight B-vitamins investigated by Denko *et al.*, was extractable from feces with water; and, it is contrary to the belief that the vitamin K synthesized by intestinal bacteria is utilizable. It is also contrary to the demonstrated function of microorganisms in ruminants (*Nutrition Reviews* **1,** *297* (*1943*)). Nonavailability, on the other hand, is suggested by the experiments of A. Williamson and H. T. Parsons (*J. Nutrition* **29,** *51* (*1945*)) who found that the urinary excretion of thiamine did not reflect the relatively large increases in fecal thiamine associated with the ingestion of plant fiber (pineapple). Similarly, a large proportion of the thiamine of live yeast cells ingested by man escapes utilization, presumably because of failure of diffusion out of the cell (*Nutrition Reviews* **4,** *45* (*1946*)).

Whether the bacterial inhabitants of the intestine are harmful or helpful to man remains obscure. The results of current investigations do not support a relaxation of efforts to improve generally the dietary supply of B-vitamins because dependence upon variable bacterial synthesis rather than upon foodstuffs is illogical, at least in so far as known nutrients are concerned. These studies emphasize the necessity of clarification of the significance of fecal excretion of vitamins in balance experiments and clarification of the relation, if any, of the intestinal flora to "conditioned" nutritional diseases in man.

PRE-EXERCISE NUTRIMENT AND PHYSICAL EFFICIENCY

The type and amount of food consumed by athletic contestants at the meal prior to competition commonly is regulated by their coaches or trainers in the belief that the physical efficiency of the contestants can be increased in this manner. A wide diversity of opinion has existed among athletic directors as to the best pre-exercise nutriment, how long it should precede the meal, and how great an increase in physical efficiency could be produced.

Few experimental investigations have been conducted to test the validity of the various training table recommendations. J. Haldi, G. Bachmann, C. Ensor, and W. Wynn (*Am. J. Physiol.* **121,** *123* (*1938*)) studied muscular efficiency in relation to carbohydrate intake. It was concluded that muscular efficiency was not related to carbohydrate oxidation at the time the exercise was taken. In a continuation of these studies, J. Haldi and W. Wynn (*Ibid.* **145,** *402* (*1945*)) observed the effect of low and high carbohydrate meals on the blood sugar level and on work performance in strenuous exercise of short duration. Swimming a 100 yard sprint was selected as a task well adapted for the evaluation of changes that might occur in the capacity to perform strenuous exercise over a short period. The performance of swimmers was the same three hours after a high carbohydrate meal as after a meal of the same isocaloric value with a low carbohydrate content. Immediately after swimming the blood sugar concentration was higher than before exercise but there was no difference attributable to the composition of the meal. Blood sugar determinations at varying times after high and low carbohydrate meals of the same caloric value without intervening exercise indicated that the amount of carbohydrate in the previous meal did not affect the blood sugar concentration.

The effect of large and small meals and small meals supplemented with 50 or 100 g. of sucrose before swimming upon the performance of the swimmers has also been studied (J. Haldi and W. Wynn, *J. Nutrition* **31,** *525* (*1946*)). Two and a half to three hours after a heavy meal, the time necessary to swim each of three laps in a 100 yard sprint was found to be the same as a similar period after a light meal. Supplementation of the light meal with 50 or 100 g. of sucrose did not alter the swimming time. The blood sugar level prior to the exercise was not affected by the previous amount of food consumed. A rise of blood sugar concentration was observed after swimming but again this did not differ in relation to the amount of food consumed. The authors concluded "there is no relationship of speed, power and skill in swimming a hundred yard sprint to the amount of nutriment taken 2 to 3 hours before swimming."

These studies are a valuable contribution to our knowledge on the relationship of nutriments prior to exercise upon the physical efficiency of the body. They indicate that in brief periods of very strenuous

exercise, muscular efficiency is dependent upon energy reserve and training and not upon the composition or the size of the pre-exercise meal. A wide variety of other energy consuming tasks could be performed after meals of different size and consumption, providing the muscular efficiency during the tasks could be measured accurately. It would be valuable to determine if pre-exercise nutriment altered the muscular efficiency during the performance of tasks involving a prolonged but less exhausting period of energy output. Such data might influence the planning of menus in industrial cafeterias. A further problem of somewhat more fundamental interest would be to determine the period of starvation necessary before there was a reduction in physical efficiency and to determine the type of meal which provided the best recovery.

ASCORBIC ACID CONTENT OF BLOOD AND PLASMA IN NORMAL RATS

The question of what constitutes an adequate functional value of ascorbic acid in human blood has been discussed widely in connection with the evaluation of nutritional status with respect to this vitamin. E. N. Todhunter and T. J. McMillan (*J. Nutrition* **31,** *573* (*1946*)) have reviewed the literature so as to compare the plasma ascorbic acid concentration in various species. They found that with the exception of a very high value for hens, average values for healthy monkeys, guinea pigs, rabbits, goats, horses, sheep, dairy cattle, and dogs maintained on adequate diets varied between 0.25 and 0.66 mg. per 100 ml. of plasma. Since rats are able to synthesize ascorbic acid, these authors felt that the plasma value in healthy rats maintained on an adequate diet would be an indication of the normal value for that species. Plasma ascorbic acid content was determined by a modification of the micromethod of R. L. Mindlin and A. M. Butler (*J. Biol. Chem.* **122,** *673* (*1938*)) and whole blood ascorbic acid levels by the method of J. H. Roe and C. A. Kuether (*Ibid.* **147,** *399* (*1943*)). The plasma ascorbic acid content was 0.87 ± 0.028 mg. per 100 ml. in 50 male rats and 0.33 ± 0.019 mg. for the 51 females. The whole blood ascorbic acid concentrations were 0.72 ± 0.017 mg. and 0.35 mg. ± 0.011 mg., respectively. The difference between the two sexes in the plasma and whole blood ascorbic acid contents was found to be highly significant. The reason for this difference was not apparent. A significant difference in hemoglobin values for the two sexes was also observed: 13.7 ± 0.10 g. per 100 ml. for males and 13.0 ± 0.12 g. for females.

The optimal plasma ascorbic acid concentration in man has been a frequent topic of discussion during the past decade. In a case of experimentally produced, uncomplicated ascorbic acid deficiency, J. H. Crandon, C. C. Lund, and D. B. Dill (*New Engl. J. Med.* **223,** *353* (*1940*)) observed that the plasma ascorbic acid concentration dropped to and remained at zero after forty-one days of the experimental regimen, even though the first abnormal clinical signs (hyperkeratotic papules) of ascorbic acid deficiency did not appear before one hundred and thirty-two days. These authors believed that the plasma ascorbic acid concentration was not a good index of experimental scurvy since it dropped to zero long before the appearance of clinical signs. The white cell-platelet ascorbic acid concentration remained measurable until just before the appearance of clinical scurvy.

L. D. Greenberg, J. F. Rinehart, and N. M. Phatak (*Proc. Soc. Exp. Biol. Med.* **35,** *135* (*1936*)) indicated their belief that plasma ascorbic acid concentrations of 0.7 to 0.9 mg. per 100 ml. were adequate but

not optimal. G. J. Kastlin, C. G. King, C. R. Schlesinger, and J. W. Mitchell (*Am. J. Clin. Path.* **10,** *882 (1940)*) suggested that plasma ascorbic acid concentrations of 0.7 to 0.4 mg. per cent indicated mild deficiencies of ascorbic acid and that concentrations of less than 0.4 mg. per cent were associated with severe deficiencies. Since these observations were applied to the spontaneous complicated forms of ascorbic acid deficiency which would be encountered in the clinic or in a nutritional survey, they are not necessarily incompatible with the findings of Crandon *et al.* in experimental uncomplicated ascorbic acid deficiency. These plasma ascorbic acid concentrations are considerably in excess of the values observed by Todhunter and McMillan in rats, a species of animal which synthesizes its ascorbic acid. This may suggest that the plasma concentrations considered optimal for ascorbic acid in man may be high or possibly that the metabolic requirement for ascorbic acid may be higher in man than in the animals which synthesize their ascorbic acid. The sex difference in plasma ascorbic acid concentrations in the rat is an interesting observation which will bear investigation in other species, especially since T. S. Sutton, H. E. Kaeser, and S. L. Hansard (*J. Biol. Chem.* **144,** *183 (1942)*) have reported a much lower urinary ascorbic acid excretion in female than in male rats.

INTERPRETATION OF BACTERIAL GROWTH INHIBITION AND ITS REVERSAL

From theoretical considerations of the kinetics of enzyme action and its inhibition, W. Shive and J. Macow (*J. Biol. Chem.* **162,** *451 (1946)*) have introduced some new terms and methods of deduction which aid in explaining the mechanism of specific growth inhibitors, and of their reversing agents, and in identifying some of the series of biosynthetic reactions necessary for growth.

To use knowledge of competitive inhibition to elucidate biochemical transformations which occur in growing organisms, the authors make three assumptions: (1) The antibacterial index (the ratio of molar concentration of inhibitor to that of metabolite which is necessary for maximum inhibition) is a property of the inhibited enzyme system which is the limiting factor for growth of the organism; (2) When the product of this specific limiting enzyme system is supplied to the organism in excess of growth requirements, the inhibitor is either rendered ineffective or affects at higher concentrations another enzyme system, for which a higher antibacterial index will be observed; (3) Precursors of the metabolite may be effective in preventing inhibition of growth by the analogue over wide ranges of concentration.

These concepts were applied to observations on the competitive inhibition of the utilization of aspartic acid by hydroxyaspartic acid. The less soluble isomer, designated as dl-para-hydroxyaspartic acid, was used. This compound was shown to possess no growth stimulating power for Escherichia coli, but on the contrary was toxic. As the amount of aspartic acid in the medium was increased, the concentration of hydroxyaspartic necessary for growth inhibition was found to increase. In the presence of sufficiently large quantities of aspartic acid even large amounts of hydroxyaspartic acid were not inhibitory. The antibacterial index remained almost constant over a wide range of concentrations. The same compound was also inhibitory for Leuconostoc mesenteroides, but with a considerably higher antibacterial index.

Further observations with E. coli showed that meso-diaminosuccinic acid inhibits growth by competition with aspartic acid, and that partial prevention of hydroxyaspartic acid inhibition is caused by panto-

exercise, muscular efficiency is dependent upon energy reserve and training and not upon the composition or the size of the pre-exercise meal. A wide variety of other energy consuming tasks could be performed after meals of different size and consumption, providing the muscular efficiency during the tasks could be measured accurately. It would be valuable to determine if pre-exercise nutriment altered the muscular efficiency during the performance of tasks involving a prolonged but less exhausting period of energy output. Such data might influence the planning of menus in industrial cafeterias. A further problem of somewhat more fundamental interest would be to determine the period of starvation necessary before there was a reduction in physical efficiency and to determine the type of meal which provided the best recovery.

ASCORBIC ACID CONTENT OF BLOOD AND PLASMA IN NORMAL RATS

The question of what constitutes an adequate functional value of ascorbic acid in human blood has been discussed widely in connection with the evaluation of nutritional status with respect to this vitamin. E. N. Todhunter and T. J. McMillan (*J. Nutrition* **31,** *573* (*1946*)) have reviewed the literature so as to compare the plasma ascorbic acid concentration in various species. They found that with the exception of a very high value for hens, average values for healthy monkeys, guinea pigs, rabbits, goats, horses, sheep, dairy cattle, and dogs maintained on adequate diets varied between 0.25 and 0.66 mg. per 100 ml. of plasma. Since rats are able to synthesize ascorbic acid, these authors felt that the plasma value in healthy rats maintained on an adequate diet would be an indication of the normal value for that species. Plasma ascorbic acid content was determined by a modification of the micromethod of R. L. Mindlin and A. M. Butler (*J. Biol. Chem.* **122,** *673* (*1938*)) and whole blood ascorbic acid levels by the method of J. H. Roe and C. A. Kuether (*Ibid.* **147,** *399* (*1943*)). The plasma ascorbic acid content was 0.87 ± 0.028 mg. per 100 ml. in 50 male rats and 0.33 ± 0.019 mg. for the 51 females. The whole blood ascorbic acid concentrations were 0.72 ± 0.017 mg. and 0.35 mg. ± 0.011 mg., respectively. The difference between the two sexes in the plasma and whole blood ascorbic acid contents was found to be highly significant. The reason for this difference was not apparent. A significant difference in hemoglobin values for the two sexes was also observed: 13.7 ± 0.10 g. per 100 ml. for males and 13.0 ± 0.12 g. for females.

The optimal plasma ascorbic acid concentration in man has been a frequent topic of discussion during the past decade. In a case of experimentally produced, uncomplicated ascorbic acid deficiency, J. H. Crandon, C. C. Lund, and D. B. Dill (*New Engl. J. Med.* **223,** *353* (*1940*)) observed that the plasma ascorbic acid concentration dropped to and remained at zero after forty-one days of the experimental regimen, even though the first abnormal clinical signs (hyperkeratotic papules) of ascorbic acid deficiency did not appear before one hundred and thirty-two days. These authors believed that the plasma ascorbic acid concentration was not a good index of experimental scurvy since it dropped to zero long before the appearance of clinical signs. The white cell-platelet ascorbic acid concentration remained measurable until just before the appearance of clinical scurvy.

L. D. Greenberg, J. F. Rinehart, and N. M. Phatak (*Proc. Soc. Exp. Biol. Med.* **35,** *135* (*1936*)) indicated their belief that plasma ascorbic acid concentrations of 0.7 to 0.9 mg. per 100 ml. were adequate but

not optimal. G. J. Kastlin, C. G. King, C. R. Schlesinger, and J. W. Mitchell (*Am. J. Clin. Path.* **10,** *882 (1940)*) suggested that plasma ascorbic acid concentrations of 0.7 to 0.4 mg. per cent indicated mild deficiencies of ascorbic acid and that concentrations of less than 0.4 mg. per cent were associated with severe deficiencies. Since these observations were applied to the spontaneous complicated forms of ascorbic acid deficiency which would be encountered in the clinic or in a nutritional survey, they are not necessarily incompatible with the findings of Crandon *et al.* in experimental uncomplicated ascorbic acid deficiency. These plasma ascorbic acid concentrations are considerably in excess of the values observed by Todhunter and McMillan in rats, a species of animal which synthesizes its ascorbic acid. This may suggest that the plasma concentrations considered optimal for ascorbic acid in man may be high or possibly that the metabolic requirement for ascorbic acid may be higher in man than in the animals which synthesize their ascorbic acid. The sex difference in plasma ascorbic acid concentrations in the rat is an interesting observation which will bear investigation in other species, especially since T. S. Sutton, H. E. Kaeser, and S. L. Hansard (*J. Biol. Chem.* **144,** *183 (1942)*) have reported a much lower urinary ascorbic acid excretion in female than in male rats.

INTERPRETATION OF BACTERIAL GROWTH INHIBITION AND ITS REVERSAL

From theoretical considerations of the kinetics of enzyme action and its inhibition, W. Shive and J. Macow (*J. Biol. Chem.* **162,** *451 (1946)*) have introduced some new terms and methods of deduction which aid in explaining the mechanism of specific growth inhibitors, and of their reversing agents, and in identifying some of the series of biosynthetic reactions necessary for growth.

To use knowledge of competitive inhibition to elucidate biochemical transformations which occur in growing organisms, the authors make three assumptions: (1) The antibacterial index (the ratio of molar concentration of inhibitor to that of metabolite which is necessary for maximum inhibition) is a property of the inhibited enzyme system which is the limiting factor for growth of the organism; (2) When the product of this specific limiting enzyme system is supplied to the organism in excess of growth requirements, the inhibitor is either rendered ineffective or affects at higher concentrations another enzyme system, for which a higher antibacterial index will be observed; (3) Precursors of the metabolite may be effective in preventing inhibition of growth by the analogue over wide ranges of concentration.

These concepts were applied to observations on the competitive inhibition of the utilization of aspartic acid by hydroxyaspartic acid. The less soluble isomer, designated as dl-para-hydroxyaspartic acid, was used. This compound was shown to possess no growth stimulating power for Escherichia coli, but on the contrary was toxic. As the amount of aspartic acid in the medium was increased, the concentration of hydroxyaspartic necessary for growth inhibition was found to increase. In the presence of sufficiently large quantities of aspartic acid even large amounts of hydroxyaspartic acid were not inhibitory. The antibacterial index remained almost constant over a wide range of concentrations. The same compound was also inhibitory for Leuconostoc mesenteroides, but with a considerably higher antibacterial index.

Further observations with E. coli showed that meso-diaminosuccinic acid inhibits growth by competition with aspartic acid, and that partial prevention of hydroxyaspartic acid inhibition is caused by panto-

thenic acid, beta-alanine, and asparagine. The antibacterial index for hydroxyaspartic acid in the presence of aspartic acid was increased sixfold by the addition of pantothenic acid to the growth medium. This change in the antibacterial index is an example of the effect of addition of the reaction product of the inhibited enzyme system to the medium. The inhibited enzyme system is no longer indispensable since the product is already present, but another enzyme system is affected, as revealed by a higher antibacterial index corresponding to this other enzyme.

Glutamic acid was as effective as aspartic acid in reversing the inhibition by hydroxyaspartic acid, except at high concentrations. Since glutamic acid could reverse the inhibition by hydroxyaspartic acid it is deduced that glutamic acid must serve as a precursor of aspartic acid. From the results with beta-alanine, asparagine, and pantothenic acid, it is concluded that aspartic acid is a precursor in the synthesis of the beta-alanine portion of pantothenic acid by E. coli. In addition, under the conditions of the measurements, asparagine is not converted to aspartic acid to any appreciable extent, and the evidence indicated that at least one additional synthetic reaction utilizing aspartic acid is prevented by sufficient amounts of hydroxyaspartic acid.

It must be remembered that the antibacterial index remains constant over a wide range of concentrations only when the other components of the medium are the same, and the size of inoculum, time, and temperature of inoculation, are not allowed to vary. Different antibacterial indexes are therefore recorded for the same inhibition in different experiments where these conditions were not held constant.

W. Shive and E. C. Roberts (*J. Biol. Chem.* **162,** *463 (1946)*) have extended the same conceptions to the interpretation of some transformations of para-aminobenzoic acid. They introduced a new term "product inhibition index," defined as the molar ratio of analogue to metabolite at which the rate of synthesis of the product is the limiting process for growth and is reduced to such an extent so as to prevent growth of the organism in a medium free of the product. As an example of the use of this term, the methionine inhibition index of E. coli for sulfanilamide is the molar ratio of sulfanilamide to para-aminobenzoic acid in a methionine-free medium which inhibits the synthesis of methionine sufficiently to stop the growth of the organism.

For a particular analogue of a metabolite in a medium free of products derived from the metabolite, the lowest product inhibition index among the indexes for all the various products is the antibacterial index. If some of the products are supplied in excess, the lowest inhibition index of the products which are not supplied becomes the antibacterial index. If all the products are supplied in excess, the analogue of course loses its power to inhibit growth. The product inhibition index, like the antibacterial index, depends on constancy of the testing conditions.

The effect of adenine sulfate on sulfanilamide toxicity for Lactobacillus arabinosus 17-5 was investigated, and the amount of sulfanilamide required for the maximum inhibition in the presence of adenine sulfate was shown to be ten times that required for maximum inhibition in its absence. The antibacterial index was raised tenfold by adenine sulfate (and no more by a mixture of adenine sulfate, guanine hydrochloride, and xanthine) under the experimental conditions used. This result indicates that the purine bases are products of metabolism produced by an enzyme system in which para-aminobenzoic acid functions.

For E. coli, the antibacterial index of sulfanilamide was raised threefold in the presence of methionine. The purine bases had no effect on the toxicity of sulfanilamide in the absence of methionine, but in the presence of methionine the addition of purines further raised the antibacterial

index tenfold. When the antagonistic effect of 4-amino-2-chloro-benzoic acid to para-aminobenzoic acid was measured for E. coli, the antibacterial index was observed to be about 3000, but in the presence of methionine this compound was unable to inhibit.

Thus the methionine inhibition index of E. coli for sulfanilamide was about one-third the purine base inhibition index, which itself was one-tenth as large as the inhibition index of another product as yet unidentified. The antibacterial index therefore varied thirtyfold, depending on the presence or absence of these two products in the growth medium. For this organism therefore, methionine and the purine bases appear to be products of enzyme systems in which para-aminobenzoic acid functions.

These observations indicate the reason why chemotherapeutic agents differ in their behavior *in vitro* from their behavior *in vivo*. The activity of an inhibitor may be overlooked by *in vitro* testing if the products of the reaction supposedly inhibited are present in the experimental medium. The authors point out that if the "effective medium" at the site of an infection lacks constituents present in the medium used for *in vitro* tests, chemotherapeutic agents of possible value may be overlooked in the *in vitro* tests; and conversely, agents which are worthless *in vivo* may be active *in vitro* if tested in mediums lacking products present at the site of an infection. Ideally, *in vitro* tests for a chemotherapeutic agent should be made in a medium resembling as closely as possible that present at the site of infection.

NUTRITIONAL EDEMA

Edematous conditions are encountered, particularly in times of famine, which are not readily explainable in terms of the hydrostatic and colloid osmotic pressures of the blood and tissue fluids and which appear to involve changes of permeability in the capillary walls, either toxic in character or of some unknown origin. A. Keys, H. L. Taylor, O. Mickelsen, and A. Henschel (*Science* **103**, *669* (*1946*)), in reviewing some of the reports from the war zones, concluded that the hypoproteinemia recorded was often slight in degree and did not relate closely to either the occurrence or severity of edema. These investigators made observations on a group of 34 men, first on a control diet, then on a European type of famine diet for six months, and finally on a "relief" diet for three months. During the famine type diet of whole cereals, potatoes, and turnips, an average of 49 g. of protein was provided, and the subjects lost an average of 24.5 per cent of their body weight. Edema appeared in some cases within two months, and in nearly all before the termination of the famine diet. The extent of edema was indicated by a relative excess of over 7 kg. of extracellular water per man, and an increase of "extracellular water" from a normal average of 282 g. per kilogram to 554 g. per kilogram.

A reduction in the plasma albumin fraction below 2.5 to 3.0 per cent (normal 4 to 5 per cent) and a total plasma protein of approximately 5 per cent (normal 7 to 8 per cent) are conceded generally to represent a "critical level" at which edema commences. Yet in these subjects with edema the decline in plasma protein concentration averaged only 0.73 g. per 100 ml. The decrease in the albumin/globulin ration was also very small compared with the change characteristic of hypoproteinemic edema. It was difficult therefore to attribute the edema to hypoproteinemia and sufficiently decreased osmotic pressure of the plasma, nor was a "reverse" osmotic pressure of the tissue fluids considered significant since "edema

fluid in this condition is extremely low in protein."

Instead of finding an increased venous pressure which might explain the edema, direct measurements actually revealed a decreased pressure during the semistarvation period. This measured an average of 4.8 cm. of saline under the adopted standardized conditions, as compared with 9.7 and 10.3 cm. measured on 12 normal subjects on two separate occasions. No evidence of a thiamine deficiency or signs of cardiac or renal failure were present. A moderate arterial hypotension was present rather than increased arterial pressure. The only remaining factor for an explanation of the edema on classic grounds would be a reduced tissue pressure, and this did appear to be subnormal in starvation as indicated by digital examination. The investigators however discount even a profound decline in this pressure as being of significance in relation to the intracapillary pressure. All of the various factors found abnormal, including the edema, responded to the return of the normal diet.

The authors conclude that famine edema is not due simply to hypoproteinemia or to renal or cardiac failure, but imply that in this condition the capillary wall is abnormal. Some factor missing in the diet would seem to be involved, but no clue is given as to its nature. A study involving partial replacements of nutrients and their effect on the edema might lead to an understanding of a condition which may be prevalent in parts of the world for some time to come.

NOTES

Letter to the Editor

Dear Sir:

We take the opportunity to correct an error in the article "Vitamin K and Tooth Decay" which appeared in the July issue of Nutrition Reviews. In the review of our article "Influence on the rat incisor of vitamin K deficiency in the presence of sulfadiazine containing diets" (*J. Dental Research* **24,** *137 (1945)*) it is said that the rats which received diets containing sulfadiazine showed "... a decrease in enamel pigmentation." In our paper we reported only "a striking deficiency of growth and a decline of eruption and attrition rates of incisors in rats on sulfadiazine-containing rations," but nothing about a decrease of enamel pigmentation.

Humberto Granados
Henrik Dam
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New Booklets

The Food and Agriculture Organization of the United Nations has established an Information Service Bulletin that will be of interest to many nutrition workers. The bulletins are distributed from the office of Sir John Boyd Orr, Director General of FAO, 2000 Massachusetts Avenue, N.W., Washington 6, D. C.

The Nutrition Division of Department of National Health and Welfare, Ottawa, Canada, has prepared a booklet, Canadian Food and Nutrition Statistics, 1935 to 1945, that includes a considerable body of data within its 22 pages.

Dr. B. S. Platt of the British Medical Research Council has issued a 41 page booklet, Special Report Series no. 253 on "Table of Representative Values of Foods Commonly Used in Tropical Countries."

A New Manifestation of Riboflavin Deficiency

R. J. Vakil (*Indian Med. Gaz.* **80,** *148 (1945)*) has made a case report from Bombay of an 18 year old girl in whom a diagnosis of riboflavin deficiency was made. The patient, after being confined to hospital with typhoid fever for five weeks complained of a "raw feeling" in the mouth, together with soreness of the tongue and difficulty in swallowing. In addition, there was complaint of "burning sensations" and discharge in the region of the vulva.

Examination revealed abnormal redness with slight crusting of the lips, especially at the angles of the mouth, and a "dark red, raw, and angry-looking tongue." A light yellow mucous discharge was seen over the vulva, which appeared congested and raw; both the urethral and vaginal orifices showed red "halves" around them. A diagnosis of riboflavin deficiency was made, and the patient was given 6 mg. of riboflavin daily by mouth, without any other treatment. Within six days, the oral and vulval lesions had disappeared completely.

The lesions of the vulva were therefore attributed to riboflavin deficiency; it was thought to be the first time that such a sign had been reported in this deficiency.

Vitamin A Tolerance Test In Dermatologic Disorders

Manifestations of vitamin A deficiency are to be found particularly in the skin. This has led various investigators to study the etiologic significance of a deficiency of this vitamin in certain diseases of the skin which are recognized as being associated with a disturbance of keratinization. As yet little in the way of objective data and no

conclusive results have been brought forth concerning the role of vitamin A deficiency in the pathogenesis of the skin disorders which have been studied. For this reason the report by D. M. Ruch, L. A. Brunsting, and A. E. Osterberg (*Proc. Staff Meetings Mayo Clinic* **21,** *209* (*1946*)) is of interest.

These investigators report the results of vitamin A tolerance tests in normal individuals and in 11 persons with various types of skin disorders. The concentration of carotene and vitamin A in the serum was determined after all food had been withheld for twelve hours. At the end of this phase of the test a massive dose of vitamin A, 7500 I.U. per kilogram body weight, was administered orally. Samples of blood were collected at intervals of two, four, six, nine, twelve, and twenty-four hours. The urine and feces were collected daily for the ensuing four days. During the study the diet was restricted to withhold sources of carotene and vitamin A. Carotene and vitamin A were measured by the method of S. W. Clausen and A. B. McCoord (*J. Pediatrics* **13,** *635* (*1938*)).

Consistent results were obtained in the normal persons although they varied within a rather wide range. The peak level of concentration of vitamin A in the serum of a normal person was reached, as a rule, six hours after the ingestion of the vitamin, but the peak level itself ranged from 1000 to almost 3000 I.U. per 100 ml. serum. It is of interest that only about 10 per cent of the amount of vitamin A given was eliminated in the feces of the normal persons.

In the cases of skin disease which included keratosis pilaris, keratosis follicularis, pityriasis rubra pilaris, ichthyosis, and congenital dyskeratosis, there was a tendency for the levels of vitamin A in the blood to be low. In 2 cases the curves for vitamin A levels were flat.

It is difficult to state what significance may be attached to this observation. A second test performed in a patient whose maximal blood level for vitamin A at the end of six hours had been 282 units in the first test, rose to 1721 in the second test. This would suggest that the massive dose of vitamin A that was administered in the course of the first test replaced a depleted store of the vitamin in the liver. Whether these observations have any relationship to the pathogenesis of the skin disorders studied is a matter for further study.

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The Nutrition Foundation was organized by food and related manufacturers in December, 1941, as a sincere expression of their interest in scientific progress and human health. The basic purposes of the Foundation are: (1) the development of a comprehensive program of fundamental research, providing basic information in the science of nutrition; and (2) the support of educational measures that will assist in making the science of nutrition effective in the lives of present and future generations.

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